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VOLUME X. STUDIES ON THE DIGESTION OF A
SEWAGE-FILTER EFFLUENT BY A SMALL AND OTHERWISE
UNPOLLUTED STREAM

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CONTRIBUTIONS

From The

SANITARY RESEARCH LABORATORY

and

SEWAGE EXPERIMENT STATION

VOLUME X

Studies On The Digestion Of A Sewage-Filter
Effluent By A Small and Otherwise
Unpolluted Stream

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Here again a new question arises, namely, To what extent will a more or less polluted or an unpolluted stream, lake or estuary receive and digest without causing a nuisance, the effluents from sewage purification works of various kinds.

It was this grave and widespread problem which was suggested to the undersigned by his associate, Professor Weston, as one worthy of careful, minute and prolonged study.

The following pages give the results of a two years consideration of a particular problem of this kind, namely the digestion by a small and rather slow stream of the tolerably well purified effluent of the sewage disposal works of a modern industrial city of 65,000 inhabitants.

Emphasis has been laid particularly upon the biological (page 4) aspects of the problem, for the reason that these have on the whole received much less attention than other aspects from students of sewage purification. By a fortunate circumstance Professor Weston was able to associate with himself in the work Mr. C. E. Turner, M.A., whose training and interests had been hitherto largely biological, with the result that the problem has been studied with unusual care from that point of view.

The results obtained by a two years' study of the stream in question prove that slowly moving water of this kind is able within the first three-fourths of a mile of its course to digest and highly purify effluents in large volume, such as it received. It appears, moreover, that, as might have been anticipated, the process of digestion is almost wholly biological; that it goes on mostly within the first mile of stream flow; and that it is extremely sensitive to conditions such as temperature, rainfall and the amount and character of plant and animal life.

If we would state in a single sentence the broad result, it may be put as follows:-- The imperfectly purified effluent of the Brockton Sewage Disposal Works is quickly and in summer almost completely purified in the small, slow moving Coweocot Stream into which it is discharged.

The Director desires to acknowledge with his thanks the faithfulness and devotion of all who have been engaged in the work, and especially the constant courtesy and helpfulness of the Officials connected with the Sewer Department of the City of Brockton. (Page 5)

STUDIES ON THE DIGESTION OF A SEWAGE-FILTER
EFFLUENT BY A SMALL AND OTHERWISE
UNPOLLUTED STREAM

By Robert Scurr Weston and C. E. Turner

(An Investigation by the Sanitary Research Laboratory of the Massachusetts Institute of Technology,--July 1, 1914, to July 1, 1916.)

While this volume is passing through the press, word comes of the death of the Anonymous Donor to whose initiative and devotion the Sanitary Research Laboratory and Sewage Experiment Station of the Massachusetts Institute of Technology owed its establishment and its support.

It will be a surprise to some to discover that our friend was a woman, and to many to learn that one who chose to select for encouragement and financial support a Sanitary Research Laboratory and Sewage Experiment Station, was a lady fond of books, of music and of painting. But those who had the privilege of her intimate acquaintance will not need to be told that our honored Donor held to the highest standards in every direction, or that her mind was akin to that which Professor Huxley has described as typical of one possessing a liberal education, "whose intellect is a clear, cold logic engine ready to be turned to any kind of work and spin the gossamers as well as forge the anchors of the mind . . . who has learned to love all beauty, whether of Nature or of Art, and to hate all vileness"---including dirt of every kind and above all sewage.

(Page 3)

INTRODUCTION

By W. T. SEDGWICK, Director

There is perhaps no aspect of the sewage purification problem more general or more important than that of the digestion of raw sewage or sewage effluents by lakes and streams of various sorts, or by estuaries, harbors or the open sea.

From time immemorial man has sought to dispose of the wastes of life by throwing them into some body of water, and as long as the amount of organic matter thus disposed of was relatively small in proportion to the size of the stream, lake or estuary, the results were fairly satisfactory. When, however, great cities seek to do likewise, and to dispose of the wastes of thousands or perhaps millions of people in this same primitive fashion, trouble almost invariably ensues.

Various means of purifying sewage either thoroughly or roughly now exist, with the result that effluents of various kinds from sewage disposal works find their way into bodies of otherwise more or less unpolluted water.

During the year 1914, Professor William T. Sedgwick, Director of the Sanitary Research Laboratory of the Massachusetts Institute of Technology, adopted a plan proposed by one of us for an elaborate study of the changes in character, and especially the biological changes, which take place in a small normal stream after this has received the effluent of a large modern sewage disposal plant.

Following a preliminary examination of several such streams, the Coweaset, a small tributary of the Taunton River (see Map), which receives the partially purified sewage from the City of Brockton, Massachusetts, having a population of 64,000, was selected as offering the most favorable conditions for study. An additional and important reason for the choice was that the City of Brockton generously tendered the privileges of its laboratory at the Sewage Beds as a Testing Station for our use.

Personnel. The work has been carried on under the general direction of Professor Weston, with Mr. Turner in charge of the Station and assisted by various trained workers, as required. R. D. Bates, '14, served as sanitary engineer until his departure for Serbia on May 12, 1915. He was followed by C. D. Bryant, '14. During the summer of 1915 E. B. Johnson, '16, and W. H. Jenkins, '16, were employed at the Station. Prior to June 1, 1915, bacterial analyses were made by G. S. Fowler. Occasional chemical analyses were made by Messrs. Kennedy and Hoyt of the Brockton Laboratory, and from time to time by A. L. Gammage of Professor Weston's private laboratory. Drawings of several organisms were made by Miss Harriet Park, a (Page 6) graduate student at the Institute during 1914. The identification of the higher plants was the work of Dr. Edmund W. Sinnott, of the Connecticut Agricultural College. To all of these our special thanks are due.

Period of Investigation. On July 1, 1914, a preliminary reconnaissance was begun to determine what analytical and field work should be done and the best methods of sampling. Before September 1st regular weekly collection of samples for chemical and biological examination and regular inspections of the banks and valley of the stream had begun. The results of a year of such records are given in the appended tables, and on them our conclusions are largely based. In addition, bi-weekly biological and monthly chemical examinations were made during a second year in order to check the accuracy of the conclusions drawn from the first year's records. It was found that the seasonal changes in temperature and rainfall for the two years were widely variant, the flow in the river being much lower when the work began than at any subsequent period; but so closely did the data under similar conditions agree that the second year's results are not included in the computations, although they have been recorded in the appended tables for reference. Limited bacteriological studies were made from March to August, 1915.

Stream and Source of Pollution. The Coweaset stream above the sewage disposal beds has an approximate flow of from 500,000 to 25,000,000 gallons per day, the low flows being greatly influenced by a mill dam just above the beds. The stream drains a sparsely settled farming region, and the section observed has only a slight slope. Less than two miles from its source it receives the effluents from the various Brockton sewage beds, and 0.73 mile below the beds it is joined by another normal stream draining an area about three times as large as that drained by the Coweaset itself. Although during the flood seasons the discharges of the two streams are

nearly proportional to their drainage areas, during periods of dry weather, when the Coweaset may contain much more sewage effluent than water, this actually discharges more than the other stream,---that is, in the ratio of 5:3. Further below the combined streams flow through a low and often marshy region until they join the outlet of Lake Mippenicket, 5.75 miles below the point of pollution.

The Brockton raw sewage as applied is very highly septicized and contains spent dyes, leather dust and other shoe factory wastes. It is pumped from a collecting well to the sewage disposal works, and the monthly average volumes treated daily during the period under study varied from 1,845,000 gallons to 2,412,000 gallons, and averaged 2,106,000 gallons daily for the year. The sewage is discharged intermittently on 37 acres of sand beds, at a rate of 60,000 gallons per acre daily, part of the sewage being first passed through a trickling filter 0.5 acre in area. Seven acres of sand surface are used for the disposal of this trickling filter effluent. At times of dry weather there is so much evaporation from the surface of the filter beds that only a part of the applied sewage finally reaches the stream.

Temperature, Rainfall and Stream Flow. The accompanying rainfall and air-temperature statistics were furnished by officials of Brockton. The water temperatures recorded during the investigation were determined weekly,---that is, when each dissolved-oxygen sample was taken.

The stream flow was measured by a weir placed just below Station 2. Readings were made on alternate days or during storms more frequently.

Sampling Methods. Bacteriological and chemical samples were collected and examined according to standard methods of water analysis. The Sedgwick-Rafter methods was used for making microscopical examinations. The ordinary method of sampling, however, was not employed but, before sampling, the deposit on the bottom and sides of the stream was thoroughly stirred with a rake in order to disperse it through the water. This method was decided upon after reconnoissances had showed that samples of the normal water contained few organisms; the majority of these had come from some distance upstream and were unlike those living on the stream bed at the point of sampling (Page 7)

Frequent sediment tests were made with the "Wizard Sediment Tester," a device used commonly for showing the amount of sediment in milk. In making this test, a pint of water is passed thru a cotton filter disc, whereupon the coarser suspended matter left behind gives a visible record of the amount and character of the plankton and suspended matter. Sediment tests were made without stirring up the bottom.

Other biological data were collected during frequent excursions along the stream, and the higher forms of plant and animal life were noted and identified.

Acknowledgements. Throughout the whole investigation, the writers have been indebted to Professor William T. Sedgwick, Director of the Sanitary Research Laboratory, both for helpful consultations and for visits in the field. They are also under obligations to other members of the Instructing Staff of the Institute for valuable advice and suggestions. Special thanks are due to the Sewerage Commissioners of the City

of Brockton; to the City Engineer, Mr. B. R. Chapman; to the Chemist in charge of the sewage disposal works, Mr. F. H. Kennedy; and to their assistants, for hearty co-operation in the work. Mr. Charles W. Johnson, Curator of the Boston Society of Natural History, has kindly assisted in the identification of many species of animals.

LIMITS OF THE INVESTIGATION AND CONCLUSIONS

Attention has been confined principally to the biological and chemical changes which take place in a small, normal stream receiving an imperfectly purified sand-bed effluent from a sewage disposal works. Changes in the flora and fauna have been noted, together with the existing correlations among various growths of organisms, the chemical character of the water, and the appearance of the stream. In making this study an attempt has been made to collect all data which might bear on the case, and although it is obviously impossible in a report to mention all the minor yet interesting relationships among conditions which suggest themselves, these can be readily traced by persons with special interests who care to study the accompanying records. (Page 8)

The portion of the stream studied--less than three miles--is strictly limited, but early in the investigation the results of analyses showed that no decided changes in the stream which were at all related to the original pollution could be discerned further down. On the other hand, changes in the first 0.73 mile below the sewage-disposal works proved to be so extensive and so rapid that it seemed best to establish sampling stations in rather close proximity throughout this critical region.

SUMMARY AND CONCLUSIONS

1. The whole study reveals the extreme importance and the energetic action of certain plants and animals in purifying a stream receiving sewage effluents.
2. The Cowequet Stream, which receives a more or less nitrified sand-bed effluent, first lays down a pollution carpet by precipitation of organic and mineral matter, including silica.
3. This false bottom contains forms of life typical of pollution and purification which furnish a better index of the true condition of the stream than do organisms found in the supernatant liquid.
4. The entrance of organic matter into the stream increases plant and animal life in a series, beginning with the lowest and ending with the highest forms, each class appearing along with a definite food material.
5. Comparison of conditions on the Cowequet with investigations of other streams, shows that the smaller, shallower and more nearly stagnant the body of water, and the more highly nitrified and clarified the effluent, the more rapid is the succession of zones of higher animal life, and the more rapid and complete the purification process.

6. In this stream, when discharging less than 5,000,000 gallons and receiving 2,000,000 gallons of sand-bed effluent daily, the most important changes occur within the first mile; below which point, biologically speaking, the stream appears quite normal.

7. The return of the stream to a normal biological condition is more rapid than its return to a normal chemical condition; and (Page 9) the rate of chemical change is much less rapid below the point where biological processes have ceased their unusual activities.

8. It appears probable from the results of this investigation that a digestive process similar to that in the Coweset River could be secured without difficulty, for an effluent line that from the Brockton sewage disposal works by dispersing it thruout a shallow pond not more than four acres in area, before discharging it into the stream.

9. Natural storage reservoirs in a stream below the point of discharge of sewage effluent are of great assistance not merely in minimizing storm effects and seasonal changes but also in restoring the water to its normal condition.

10. In winter, indications of pollution extend farther down the stream than in summer. In other words, the critical or digesting region is elongated.

11. Although very slight changes are to be noted in winter, in summer, when biological activity is at its height, the 0.7 mile stretch of stream below Station 2, providing a normal storage period equivalent to about 1 hour's flow, effected, on an average, the removal daily of 39.5 lbs. of total nitrogen, 10.1 lbs. of nitrogen as albuminoid ammonia, and 101.3 lbs. of the carbonaceous organic matter as measured by the oxygen-consumed test.

12. In this stream the dissolved oxygen tends to decrease until the excessive growth of animal life, favored by pollution, ceases. This factor outweighs both the normal re-aeration of the stream and the oxygenating effect of plant growth.

13. The carbon dioxide introduced with the effluent tends to decrease down stream, at first rapidly and afterwards very slowly.

14. The rate of the oxidation of nitrogenous compounds varies with the season, and with the intensity of organic growth; it is very rapid in summer and hardly discoverable in winter.

15. The types of organisms which most clearly indicate the degree of pollution are Bacteria, Protozoa and Diatoms.

16. Certain other types of higher organisms are characteristic of pollution, but in general the higher the type and the more varied its food supply the less reliable the form as an index of pollution. (Page 10)

17. The following organisms are commonly found in the polluted part of the stream:----

Bacteria: B. coli and allied forms, Cladothrix.

Water Mould: Leptomitius

Diatoms: Navicula forms, Gomphonema, Meridion.

Protococcales: Protococcus.

Desmids: Closterium.

Filamentous Algae: Spirogyra communis, Tribonema (Conferva).

Phanerogams: Potamogeton, Sparganium, Sagittaria, Polygonum, Glyceria.

Protozoa: Amoeba, Arcella, Chlamydomonas, Euglena viridis, Monas, Carchesium, Colpidium, Euplotes, Lionotus, Loxophyllum, Metopus, Oxytricha, Vorticella.

Bristle Worms: Nais, Tubifex.

Rotifers: Rotifer, Diglena.

Leeches: Erpobdella punctata, Glossiphonia stagnalis.

Snails: Planorbis trivolvis, Physa heterostrophus.

Crustacea: Asellus communis, Cyclops, Pleuroxus, Simocephalus.

Insect Larvae: Chironomus decorus, Ptychoptera.

18. Most of the organisms occur in "blooms" which are subject to fairly regular seasonal variations.

19. Chemical changes in the stream are due to bacteria more than to any other group of organisms. The higher animals are important by removing an excess of lower organisms.

20. The rate of decrease of bacteria is most rapid at the point of entrance of the effluents, and except during the winter floods very slow below the first mile.

21. Diatoms are more abundant in the normal stream, but certain forms can live in highly polluted water.

22. The algae are increased in quantity by the entrance of the effluent. Certain forms are shown to have a preference for polluted and others for unpolluted water. (Page 11)

23. The water mould, Leptomitius, has been found when oxygen was plentiful and the alkali and nitrate producing bacteria fewer and less active.

24. Different species of higher plants will tolerate pollution in different degrees. They are important in holding back suspended matter and preventing small organisms from being washed down by the current; they also assist in the removal of colloidal suspended matter and in checking the flow of the stream.

25. Abundance of Hydra is apparently correlated with the abundance of small crustacea.

26. Large numbers of rotifers and small crustacea may indicate the presence of simple green algae and not necessarily of sewage pollution.

27. Certain worms and insect larvae assist materially in the digestion of the debris accumulated in the pollution carpet.

28. Fishes (trout, suckers) were occasionally observed, even in the most polluted part of the stream.

DATA, RESULTS AND DISCUSSION

Description of the Brockton Sewage Disposal Works. The sewage of the City of Brockton is first screened and then pumped to the disposal works, some of the sewage being sixteen hours old when it reaches the filter plant. Even though 650,000 gallons of sewage daily are given a preliminary treatment on the trickling filter, the 37 acres of sand beds are overworked and the effluents are not as good as those produced in former years. The chief difficulty is the lack of oxygen to oxidize a strong septic sewage, especially in winter when the beds cannot be cleaned frequently. During the year beginning September 1, 1914, the effluent from the 15-inch drain, which is typical of the discharge of all the filters, had the following average composition. (Page 12)

MONTHLY AVERAGE ANALYSES OF EFFLUENT FROM 15-INCH DRAIN AT BROCKTON, IN PART PER MILLION

Date	Free Ammonia	Albuminoid Ammonia	Nitrates	Nitrites	Oxygen Consumed	Dissolved Oxygen Saturation
1914						
Sept.	29.62	1.6	1.87	.039	15.9	18
Oct.	27.48	1.4	3.20	.052	15.4	23
Nov.	26.09	1.9	.00	.070	22.7	24
Dec.	32.29	2.6	4.50	.007	25.9	21
1915						
Jan.	29.64	1.6	.14	.005	22.1	33
Feb.	22.67	1.4	.09	.025	18.4	35
Mar.	24.07	1.6	.00	.014	19.4	28
Apr.	21.31	1.7	.14	.037	20.7	29
May	25.04	1.4	.42	.066	18.3	32
June	30.00	1.5	6.66	.104	18.5	33
July	27.98	1.4	.26	.058	17.6	22
Aug.	25.54	1.7	1.54	.10	18.9	24
Sept.	26.12	1.6	.30	.025	20.1	24

These analyses do not show the excessive amounts of iron contained in the effluents.

Because the beds are overworked and because they suffer a great deal from sub-surface clogging, the effluent varies greatly in composition from time to time. This is particularly noticeable before and after the beds are cleaned, during which period large amounts of organic matter have to pass through the beds in an only partially purified condition.

These discharges of organic matter cause marked and relatively sudden increases in the pollution of the Coweaset Stream, as shown particularly by the oxygen consumed, the nitrogen as albuminoid ammonia and by the bacteriological analyses. (Page 13)

GENERAL DESCRIPTION OF THE COWEASET STREAM

The various sampling stations were located as follows:---

<u>Station No.</u>	<u>Location</u>
1	Immediately above Beds
2	Immediately below lowest drain.
3	980 feet below lowest drain.
3A	2,000 feet below lowest drain.
4	3,650 feet below lowest drain.
5	11,580 feet below lowest drain.
6	19,700 feet below lowest drain.
7	On tributary below Station 4.
8	On Daley Brook, above Beds.

Station 3A was established after it was found that changes in the first 0.75 mile below the sewage disposal works were unexpectedly marked and rapid.

On May 12, 1916, data regarding the rate of stream flow between Station 2 and 4 were collected with the results shown below. A sack filled with shavings was used as a float, being 60% submerged at the beginning, and gradually sinking until it was floating just under water at the end of the experiment.

Time of Stream Flow.

Stations 2 to 3	8 min.
Stations 2 to 3A	22 min.
Stations 2 to 4	51 min.

A careful study and survey of the stream, made in August, 1914, at the beginning of the investigation, revealed its general characteristics. At Station 1 the water is the ordinary, fairly clear, brown water of the region. The bed of the stream is sandy and the vegetation normal. Daley Brook enters below Station 1. This brook receives some sewage effluent; in summer its bottom is covered with a thick brown growth of Crenothrix. Below the confluence, the stream serves as the effluent channel between two groups of sewage disposal beds, and at periods of low flow contains at least 80 per cent. of sewage effluent. There is now a marked change in the nature of the bed of the stream. It consists of dark-colored ooze, a "pollution carpet," made up of humus matter precipitated from the effluent, together with other organic and mineral debris. This type of stream bed continues nearly to Station 4, though the general appearance of the (Page 14) river is changed by the markedly greater number of higher plants which, practically absent at Station 2, increase gradually in luxuriance and in variety. There is also an increase in the number of such animals as worms, molluscs, crustacea and insects.

The addition of the tributary stream of purer water (p.6) causes a marked change in appearance, for while the deposits of ooze continue for a short distance below the confluence, the stream bed soon becomes firm again and either sandy, clayey or peaty in character, according to the nature of the soil through which the stream flows. The growth of aquatic plants, while unusually abundant, is no longer as "rank" as it was at Station 4. Below Station 5 the river deepens and acquires all the usual characteristics of a stream flowing through swampy land. The map shows both the low and high water limits of the small stream. At many places there are wide stretches of tussock (i. e. boggy) marsh, but where the slope is steeper the brook flows between firm banks.

The colored water, clear at Station 1, is turbid at Station 2. This turbidity is rapidly decreased during the summer when the normal flow is low and the effect of contact with aquatic plant is greatest; and at these times the plant stalks are coated with humus-like matter removed from suspension. Below where the Coweeseet is diluted by the tributary, the water is still slightly abnormal in appearance, due to the small amount of suspended matter remaining in it. Nothing unusual however, would be noticed by a casual observer, either in the water itself or in the fauna and flora.

During the fall the stream is seen at its best. Afterwards the aquatic plants disappear and with them their beneficial effect upon the appearance of the stream, which, however, alters but slightly in appearance until December, when the water mould, *Leptomit*, appears---first near the effluent outlets and later spreading over the banks and bottom of the stream in a thick mat which extends as far as Station 4 and soon becomes covered with a precipitate of organic debris. (Page 15)

Coincident with the occurrence of *Leptomit* the velocity and volume of the stream gradually increase. This greater dilution should improve its appearance, but such is not the fact because the growths of *Leptomit* break down and the detached portions, together with the liberated debris, having less opportunity to subside, are carried along in the now rapidly flowing current.

During the cold weather there is no change in the appearance of the stream between Stations 2 and 4; consequently the river at the latter point is distinctly inferior to its tributary, while in the summer they are more nearly alike. There is, therefore, greater improvement below Station 4 in winter than in summer.

During the flood season the banks overflow and growths of *Leptomit* are abundant everywhere. With the advent of warmer weather large masses of algae appear along with the *Leptomit*. These growths are first noted in the region of Station 4; later they appear further upstream. As the waters recede, the organisms die and a black residue, resembling in appearance sewage scum, is left behind. The *Leptomit* itself disappears from the main stream early in summer; there is then a rapid increase in plant and animal life, and all of the conditions described at the beginning, recur.

The winter changes in the appearance of the stream are as though the zone of greater pollution, characterized by the presence of *Leptomitus*, extends itself down the stream only to withdraw in the spring, being succeeded by growths of algae, higher plants and animals.

CHEMICAL CHARACTER OF THE COWEESSET RIVER

Tables. The results of chemical analyses, expressed as parts per million, are given in Tables 35 to 44. Monthly averages of the results of individual determinations have been computed and are given in Tables 3 to 12. Monthly averages expressed as pounds per day, in Tables 21 to 28. (Page 16)

Discussion of Results. The character of the stream, as shown by the individual determinations at the various times, and under the varying conditions of stream flow, season, etc., are discussed as follows:

Carbon Dioxide. The carbon dioxide results are very consistent. At Station 1 (above the beds) the carbon dioxide reached two high points during the year, in December and July, respectively, and fell to correspondingly low points in May and September. This in general was the seasonal variation observed also at Station 7 and to a slightly less degree at Stations 5 and 6. The seasonal fluctuations of CO₂ are therefore greater in the normal than in the polluted stream.

After the sewage effluent is added, the normal seasonal characteristics are obliterated and the amount of carbon dioxide is related to the character of the sewage effluent and the degree of its dilution by the stream; the carbon dioxide is much higher during the low water than during the high water period of the year.

The removal of carbon dioxide from the stream below the point of pollution is continuous, being most rapid at first and later very slow. For example, in the 0.7 mile between Stations 2 and 4, the carbon dioxide is reduced by an average of 14.4 parts per million. The stream is then diluted by the tributary and in the mile between Stations 5 and 6 the carbon dioxide is reduced by an average of only 1.5 parts per million.

The percentage removal of carbon dioxide is greater in summer than in winter and is at its minimum during the spring season when the organic matter accumulated during the winter is decaying rapidly. It is apparent that the rate of reduction of carbon dioxide in the stream is the product of two factors,--first, the physical effect of aeration which tends to reduce the amount in solution, and second, the effect of plant growths which consume carbon dioxide and liberate oxygen. While it is true that carbon dioxide is produced at all time by bacterial and animal growths, and at night by plant growths, this production is overbalanced at all points by the two factors last mentioned. Because (Page 17) bacterial growth is most intense at Station 2, it is probable that the greatest evolution of carbon dioxide occurs at this point.

Dissolved Oxygen. As in all streams, the amount of dissolved oxygen varies inversely with the temperature, being lowest in summer and highest in winter.

The addition of sewage effluent causes a sudden decrease in the amount of dissolved oxygen at all seasons and this amount is progressively reduced as far as Station 4. Then the inflow of the tributary causes a marked increase in the oxygen, but at Station 5 the amount is still lower than at Station 1; and while on an average there is an increase between Stations 5 and 6, this is hardly perceptible and, because the stream flows quietly through marshy land, it is probable that a long time is required to restore the original oxygen content.

The chief factor, however, affecting the dissolved oxygen content is dilution with effluent poor in oxygen. This dilution is greatest in winter when the effluent is least purified and least in summer when the effluent is best purified. On an average the stream is 60% saturated with oxygen at Station 2 and 40% at Station 4. In February, however, the water at Station 4 is 68% saturated and in August only 12% saturated. Notwithstanding the low oxygen content during August, the water is better in appearance and also in some other respects than when the oxygen present and the dilution are highest. And this is due to biological purification, particularly between Stations 2 and 4.

On September 1, 1915, determinations of dissolved oxygen and carbon dioxide were made at sunrise, noon and sunset, respectively, in order to learn the effect of the growing vegetation upon the gas contents of the water. Near Station 3A samples were taken from a stagnant pool connected with the stream, which was green with *Chlamydomonas*, *Euglena* and other algae. The results are given in the accompanying table and show both the degree to which the carbon dioxide is increased and the oxygen reduced during the night, and vice versa during the day.

For the sake of comparison, similar samples were collected from the stream itself near Stations 2 and 4. on April 20, when (Page 18) there was no plant growth in the stream, and on June 29 when it was abundant. The results show that the changes taking place in the main stream, although less intense, are similar to those in the stagnant pool, with the exception that during the growing season the carbon dioxide is not reduced during the day time corresponding to the increase in oxygen. The explanation of this probably lies in the fact that carbon dioxide was being produced by the fermentation of organic matter on the bottom of the stream.

Comparison of the April and June results indicates that the oxygen increase was entirely due to plant activity, as the following table suggests.

SPECIAL EXPERIMENTS TO SHOW VARIATIONS IN DISSOLVED
OXYGEN AND CARBON DIOXIDE

Date	Time	Dissolved P.P.M.	Oxygen % Saturation	Carbon Dioxide P.P.M.	Temperature Co
<u>Samples taken at Dam below Station II</u>					
Apr. 20	8.00 A.M.	8.84	75.3	14.0	8.5
	5.30 P.M.	7.83	74.8	11.2	13.5
June 29	5.00 A.M.	5.48	57.4	22.6	18.0
	12.00 M.	5.54	62.1	21.7	21.5
	5.00 P.M.	5.35	61.4	23.0	22.0
<u>Samples taken at Station IV</u>					
Apr. 20	8.00 A.M.	7.10	60.2	11.7	8.25
	5.30 P.M.	6.72	64.9	10.7	11.0
June 29	5.00 A.M.	3.27	34.4	20.5	18.0
	12.00 M.	4.60	51.6	20.5	21.5
	5.00 P.M.	3.40	38.5	22.0	22.0
<u>Samples taken from Pool near Station III</u>					
Sept. 1	Sunrise	1.24	12.1	32.9	14.5
	12.00 M.	5.55	60.5	26.8	20.0
	4.30 P.M.	5.88	62.9	26.2	19.0

(Page 19)

Oxygen Consumed. The amount of oxygen consumed in the unpolluted water (Station 1) was normal, being lowest in the late fall and highest in May and June.

There are three main factors which determine the amount of oxygen consumed at Station 2, namely the amount of carbonaceous matter in the unpolluted water, the amount in the effluent, and the flow of the stream; the volume of effluent is practically constant and does not materially affect the result.

At Station 2 the highest oxygen consumed occurred in the month of July, 1915, with other high points in January and May. Each of these months shows a rainfall markedly greater than that of the preceeding month, and the month of May, moreover, is the time when the surface of the filter beds is disturbed most by the spring cleaning. The oxygen consumed was lowest in September, 1914, when the effluent reached its highest degree of purification,---high enough to overcome the effect of the lowest dilution which occurred at that time.

For determining the effect of self-purification, the individual oxygen consumed results are not so regular as are, for example, those for dissolved oxygen or carbon dioxide. Nevertheless, they show quite accurately the condition prevailing in the stream. They show a reduction in carbonaceous matter between Station 2 and Station 4, except during February, March and April. During this period the bottom and sides of the stream were covered with a dense growth of Leptomitius, and the decomposing fungus and the constant liberation of materials collected with it greatly increased the amount of suspended matter in the lower stream, thereby increasing the oxygen consumed. This effect is especially noticeable following storms, when the amounts of suspended matter of all kinds are greatly increased above those observed during rainless periods. Leptomitius decomposed and practically disappeared before June, and when it was absent there was a reduction in oxygen consumed between Stations 2 and 4. However, it is probably not true that the lack of reduction is wholly due to the presence of the fungus, itself an index of less rapid purification by bacteria. (Page 20)

While the results show an average reduction of oxygen consumed from 13.14 parts per million at Station 2 to 11.90 parts per million at Station 4, they show no uniform reduction between Stations 5 and 6. Nevertheless, the oxygen consumed at Station 5 has nearly reached the normal for the region; consequently only slow reductions can be expected farther down, especially when the constant inflow from the marshy valley below Station 5 is taken into consideration.

Turbidity. The normal stream is practically clear. The turbidity increases at Station 2 and during the months when Leptomitius is present and is decomposing there is a further increase between Stations 2 and 4. It is to be noted in this connection that while Leptomitius practically dies out before June, the deposit which it leaves continues to decompose and pass down the stream for about a month longer. From July to October there is a rapid reduction in turbidity between the above stations. Monthly averages show a fairly consistent reduction between Stations 5 and 6.

Color. The Coweaset river water has the brown color which is characteristic of the waters of the region. The annual average color is 71 parts per million.

Owing to the staleness of the Brockton sewage, there is considerable reduction of iron in the beds, and while the effluents are fairly clear when first discharged, iron is precipitated in samples after standing. The presence of this iron affects the color of the river water, especially at Station 2, where it is highest. Below Station 2 there is a reduction in color during those times of the year when the greatest opportunity exists for oxidation and precipitation. During the flood periods, when vegetation is least abundant and when there is consequently little or no precipitation, the color is not reduced between Stations 2 and 4. At all times the color is reduced to normal before Station 5 is reached.

Total Nitrogen. The total nitrogen in the unpolluted stream is normal, increasing with the advancing season and decreasing again until another spring. (Page 21)

The total nitrogen in the polluted stream at Station 2 is greater in amount than, though roughly parallel to, the total nitrogen at Station 1. From the time the Leptomitus has reached its maximum growth until it and the deposit accumulated by it have entirely disappeared, little or no reduction of total nitrogen takes place between Stations 2 and 4. During the warm weather, however, the total nitrogen is reduced 30 per cent. in the one hour flow period between these stations, making a corresponding yearly average reduction of nearly 0.5 part per million, or about 20 per cent.

The results at Stations 5 and 6 are of no great significance because of conditions peculiar to the stretch between these stations. These conditions probably obscure or overweigh the slight removal of total nitrogen which one might expect below Station 4 during part of the year. On the average there is little difference in total nitrogen between Stations 5 and 6.

Free Ammonia. The free ammonia at Station 1 was low for most of the time, rising to 0.1 part or more on several occasions, and on July 19, 1915, to 0.66 part per million.

The source of the free ammonia at Station 2 is of course the effluent from the disposal beds, and because it is diluted with varying volumes of stream water, the results at Station 2 are not very constant. From August to January, inclusive, there is a slight reduction of free ammonia between Stations 2 and 4 under average conditions, although individual sets of samples show marked variations from the average. Generally speaking a like effect occurs between Stations 5 and 6.

Apparently the free ammonia is less affected by the biological factors of the stream than is any other determined constituent and therefore, is modified chiefly by the dilution in the short time elapsing as it flows by the various sampling stations.

Albuminoid Ammonia. The albuminoid ammonia results closely parallel those for total nitrogen; they are more sensitive, however, to small changes in the character of the water. There is a consistent reduction through the summer and apparently a slight reduction in winter when the stream is not disturbed by (Page 22) meteorological conditions. The yearly average shows a reduction between Stations 2 and 4 of .039 part per million, or 6 per cent. The average reduction for the months of June to September, 1915, was .303 part per million, or 30 per cent.

Nitrogen as Nitrites. The normal stream is practically free from nitrites but large amounts of these are introduced with the effluent above Station 2. Below this latter point the usually increasing amount of nitrites are evidence of increased activity of the nitrifying or denitrifying organisms.

Nitrogen as Nitrates. The nitrates in the normal water are less than one part per million at all times, being highest when, in the fall, the river is fed most largely from ground water sources. Two types of organisms tend to reduce the amount of nitrates,---first, the green plants and, second, the denitrifying bacteria which abound in the stream bottom whenever there is a considerable accumulation of organic matter.

The failure of the stream to show at certain seasons the progressive increase in nitrates which the nitrite figures would lead one to expect, is probably due to one of these factors.

DIAGRAMS (Diagrams appended)

For convenient study, certain of the results have been plotted in Diagrams, a list of which is as follows:---

- I. Yearly average carbon dioxide, dissolved oxygen, oxygen consumed and color at Stations 1 to 6, inclusive.
- II. Yearly average nitrogen as free ammonia, albuminoid ammonia, nitrites and nitrates, also total nitrogen, at Stations 1 to 6, inclusive.
- III. Yearly average number of Rotifera, Diatoms, Infusoria and Bacteria,---the latter determined by 24-hour counts on agar at 37.5° C., at Stations 1 to 6, inclusive.
- IV. Average monthly total bacteria and acid colonies on agar, for the 6 months ending September 1, 1915.
- V. Average monthly carbon dioxide at Stations 1, 2, 4, and 5, for the year ending September 1, 1915. (Page 23)
- VI. Average monthly dissolved oxygen at Stations 1, 2, 4, and 5, for the year ending September 1, 1915.
- VII. Average monthly color at Stations 1, 2, 4, and 5, for the year ending September 1, 1915.
- VIII. Average monthly oxygen consumed at Stations 1, 2, 4 and 5, for the year ending September 1, 1915.
- IX. Average monthly nitrogen as free ammonia at Stations 1, 2, 4, and 5, for the year ending September 1, 1915.
- X. Average monthly nitrogen as albuminoid ammonia at Stations 1, 2, 4 and 5, for the year ending September 1, 1915.
- XI. Average monthly nitrogen as nitrites at Stations 1, 2, 4, and 5, for the year ending September 1, 1915.
- XII. Average monthly nitrogen as nitrates at Stations 1, 2, 4 and 5, for the year ending September 1, 1915.
- XIII. Average monthly turbidity at Stations 1, 2, 4, and 5, for the year ending January 1, 1916.
- XIV. Average monthly organic nitrogen at Stations 1, 2, 4, and 5, for the year ending Feb. 1, 1916.
- XV. Temperatures of Stream at Stations 1, and 2, for the year ending September 1, 1915.

XVI. Average monthly stream flow and total rainfall for the year ending September 1, 1915.

GENERAL BIOLOGICAL STUDIES

Until recently, comparatively little work has been done in correlating biological phenomena with definite kinds of pollution. In Germany the relation of biological activity to chemical changes in water has been studied by Marsson, Kolkwitz, Wilhelmi, Spitta and others; while in America the investigators include Sedgwick, Jordan, Mrs. Richards, G. C. Whipple, Bunker, M. C. Whipple and the officers in Government departments, particularly in the U. S. Public Health Service.

Probably the earliest studies relating to this line of work were those of such men as Ehrenberg and Cohn upon the fauna and flora of polluted waters of various sorts. The Massachusetts State Board of Health, in its classical earlier reports, describes many of the organisms, chiefly bacteria, associated with sewage pollution. Then Marsson's work outlined the parts played by the various groups of organisms in polluted water and mentioned many types which appeared as well as what may be called the sequence of forms. Kolkwitz and Spitta paid more attention to particular forms and the recent work of Wilhelmi is in the main a catalogue of types listed, as well as possible, according to the degree of pollution in which they were found. G.C. Whipple's report to the City of Rochester, N. Y., 1911, as well as other American data, shows the successive increase of organisms below regions of stream pollution. All of this work, as well as that done by the U. S. Public Health service on the Illinois, Potomac and other streams, has been carried on with a more direct type of pollution and with materially larger bodies of water. No previous work, as far as known, deals with the process of digestion of a reasonably clear effluent or follows in detail the seasonal changes in the chemical, physical and biological character of a polluted stream, or compares the relative efficiencies of physical, chemical and biological purification factors.

Certain facts are known at the outset from general biological information. For example, the work done by saprophytic groups, including bacteria and higher fungi, which abound where organic food is plentiful, and the details of the steps in processes of mineralization or nitrification, are well understood. The higher forms may be divided roughly into two general classes; the food producers, or green plants, and the food consumers, or animals. With an increase of the nitrogenous and carbonaceous material in a stream, the algae multiply, consuming large amounts of carbon dioxide and liberating nascent oxygen which aids in purification. The abundant bacteria are fed upon by protozoa and both these and the algae are eaten by higher forms, including a succession of rotifers, crustacea, molluscs, insects and fishes.

The following are some of the questions which this research has sought to answer. What definite changes take place in a stream when it receives the partly purified sewage effluent from slow sand filters? For how great a distance can the polluted condition be traced through biological findings?

To what extent are biological conditions influenced by season or temperature, the changing chemical character, the intensity of pollution and the sequence of organisms? In answering these questions, an attempt has been made to give a biological as well as a chemical picture of the stream, reducing where possible all data to quantitative terms. Many relations have been suggested which cannot be discussed in detail and the need for further physiological studies upon organisms inhabiting both pure and polluted water is recognized. By such means, the cause of the appearance of certain organisms and their significance can be best ascertained.

The kinds of biological data collected may be grouped under three headings---bacterial counts, microscopical counts of other micro-organisms, and information gathered from surveys involving the identification of the larger plants and animals, together with studies of special samples. The bacteriological studies have been limited and greater emphasis placed upon the higher forms of life, the chief source of data being the microscopical examinations.

The samples for these examinations, taken weekly throughout the first year, were 500 cubic centimeters in volume and collected after disturbing the bottom of the stream. While the accuracy of this method is subject to a variation of perhaps 20% and the (Page 34) individual samples are necessarily widely varying, its results are in concrete terms and numerically expressed.

It was desirable, in making the investigation, to find the types and numbers of organisms "working" at the different points along the stream. A study of surface samples would not give this information and because the stream is not more than eight or ten feet wide, it was decided that the best expedient would be to disturb the bottom and sides over a given area with a rake, and to collect the sample from the clouded water produced thereby. In collecting the sample, an ordinary garden rake with a wide-mouthed, 500 cubic centimeter bottle tied to the end of the handle, was used. The rake was drawn across the stream and the sample collected immediately from the clouded water, at a depth of from six to twelve inches below the surface. This sample was concentrated to 5 or 10 c. c. for counting. Naturally somewhat less consistent microscopical results are obtained by this than by the usual method, but because the sample is taken from the upper strata of water the results, though varying, cannot overestimate the actual number of organisms per cubic centimeter.

Limitations of space make it impracticable to publish the detailed results of individual determinations. Consequently, a table showing the monthly averages of these has been prepared, and the original data placed on file in the Department Library of the Institute. This table includes all the forms which were revealed by the microscopical examination excepting those which were seen but once. All forms except the filamentous plants are recorded as numbers per cubic centimeter, and these are recorded in terms of standard units per cubic centimeter. The standard unit,---20 micra on a side,---is measured by a micrometer square, and is necessarily recorded with a less degree of accuracy. Statements of the total number of crustacea, diatoms, rotifers and infusoria follow the respective groups. In the latter case the figures do not agree with the totals of individual records for the reason that a special enumeration of the number of infusoria and their volume in standard units was made upon first observa- (Page 35) tions of a

new microscopic field. An accurate record of the total number of ciliates and colorless flagellates was thereby obtained, altho in most cases their rapid movement made it impossible to identify all the individuals first seen.

An examination of the above mentioned table will reveal a fact, which was also apparent from frequent surveys of the stream, namely, the cyclical appearance of organisms of most plant and animal groups in what may be called a "bloom." Individual types are observed first in small numbers, then rapidly increasing until a high point is reached and then disappearing, sometimes for a long period. This probably does not mean that the form has entirely disappeared, but that the numbers are too few to be conspicuous.

Since organisms must occur with a frequency of one per cubic centimeter or one thousand per liter in order to appear consistently, it is obvious that many of the rarer forms would not be included in the determinations. Some of these forms have been found outside of the routine samples and are mentioned in the text, while doubtless many have been overlooked entirely.

The microscopical counts, during the second year of the investigation, were made bi-monthly and because of this, as well as the greater rainfall during that period, the monthly averages were somewhat lower. The difference, however, is one in quantity and not in kind.

The original data regarding identifications have been kept, and these, together with preserved specimens of a large proportion of the forms, are available should anyone desire to look up further details regarding any special organism. A brief discussion of each group follows.

SCHIZOPHYTA

Bacteria

The work in bacteriology has been confined to the enumeration of the total number of colonies growing on agar incubated for 48 hours at 37° C., the total number on agar for 72 hours at 20° C., and the acid colonies on litmus-lactose agar incubated (Page 36) for 48 hours at 37° C. Samples were plated bi-weekly or weekly between March 24 and August 9, 1915, except for the 20-degree counts which could not be continued after May 21st.

The relative numbers of bacteria at the various stations are shown by Tables 29 to 34 and Diagram No. III which bring out certain facts clearly. In connection with the expected increase in numbers of bacteria at the point of pollution and their subsequent consistent reduction, it is interesting to note that for the three months (March to May) in which the 20-degree counts were made, their percentage increase was as great as that of the 37-degree forms, the increase being 201% in both cases. This condition would not be expected from a grosser pollution than a sand bed effluent. Proof of this is found in the effect produced on the stream by cleaning the beds, which necessarily involves a disturbance of the filter surface. In 1915, most of this work was done in May and the rise in the numbers of bacteria growing at 37 degrees, shown so clearly in Diagram IV, furnishes a direct index of the reduced bacterial efficiency of the filters.

Whereas for the months of March and April the average increase in bacteria growing at 20 degrees was 342,000 per c. c., and in those growing at 37 degrees, 79,000; during the month of May the average increase in those growing at 20 degrees was only 294,000, while that in those growing at 37 degrees was 612,000.

The data also show the reduction in numbers to be most rapid nearest the point of pollution. This may be seen more readily by the following figures, which give the average decrease in numbers of 37-degree bacteria per c. c. between different points in the stream.

Stations	2-3	3-3A	3A-4	4-5	5-6
Decrease in number per 100 lineal feet,	2250	1200	500	65	43

It may be noted that often in summer as many organisms are found at Station 6 as at Station 5, and that at both of these stations there are more bacteria than at Station 1. These last two (Page 37) facts are explained in part by the inflow which the lower section of the stream receives from pastures and cultivated lands.

Seasonal changes are also marked. Diagram IV. shows both reduction of 37 forms in summer as the filter beds become more efficient, and the seasonal changes in the number of acid forming bacteria. The latter steadily decrease in number as the warm weather advances and the alkali producing types predominate. In other words, more active nitrification begins in the stream. This is shown chemically by the increase in free ammonia.

The three genera of higher bacteria found, *Crenothrix polyspora*, *Cladothrix* and *Beggiatoa*, were not studied biochemically, but the identification was made on the basis of morphology.

Crenothrix is a common form appearing from June to December. In many places near the filter beds it covers the stream bottom with a brown, gelatinous mass. It becomes less abundant farther down the stream and has never been found below Station 4 or in the unpolluted water. This is in spite of the fact that the amount of iron increases below the point of pollution, due to deposits in the soil, where iron exists in combination with organic matter, and is higher at Station 5 than where *Crenothrix* is most abundant. Its seasonal appearance is at present not accounted for. The results of a set of determinations of total iron are as follows:---

RESULTS OF THE DETERMINATION OF IRON IN SAMPLES

COLLECTED FROM THE VARIOUS STATIONS ON JULY 6, 1916

Station No.	1	2	3	3A	4	5	6	7
Parts of Iron pr. mill	0.85	2.30	2.85	2.85	3.90	3.60	1.98	0.78

Cladotrix (Sphaerotilus) occurs at practically all seasons of the year, being somewhat more abundant at the time of greater pollution. It is ordinarily found only between Stations 2 and 4, but in winter, following storms and at periods of high stream flow, it has been found at Stations 5 and 6 as well. It furnishes a qualitative index of pollution. (Page 38)

Beggiatoa was found frequently, both in the polluted and unpolluted parts of the stream, being more abundant in the former regions, especially in winter when higher vegetation is absent.

Cyanophyceae

The only blue-green alga occurring in the samples is Oscillatoria. This is found both in the polluted and unpolluted parts of the stream, being more abundant in summer than in winter. Two species have been identified,---Oscillatoria percurse and the less common Oscillatoria subtilissima.

EUMYCETES

Only two types of higher fungi were found in the stream. These are Leptomitius lacteus, Agardh, and a segmented form resembling Penicillium, the latter occurring but rarely in the samples from Stations 2 and 6 and only as microscopic particles. Leptomitius, on the other hand, is very abundant and has a marked influence on the appearance of the stream. It has a sharply defined seasonal distribution, appearing about the middle of December near the mouths of the effluent drains and rapidly accumulating in the stream as far down as Station 4, the point of heaviest growth moving down and stopping near Station 3A. The bottom and sides of the river are then thickly covered with a white growth which soon becomes dirty brown from an accumulated mass of material which has settled out from suspension and attaches itself to the Leptomitius. There are literally tons of this Leptomitius in the stream. Great quantities are washed out by heavy storms and after the growth has reached its height, small detached, floating particles of it may be seen at all times. In spite of this, it never occurs below the affluent stream excepting as small particles which have lodged there and persist for a time, and less than half a mile below the point of confluence it disappears entirely.

On January 9, 1915, a mass of Leptomitius 60 c. c. in volume, was examined to discover what organisms were present with it. Three crustacea (Asellus) were found, while midge larvae (Page 39) (Chironus decorus) and naid worms were present, about one per c. c. in number. The principal unicellular forms were:---

SPECIES	<u>Protozoa</u>	NO. PER C. C.
Colpidium		75
Chlamydomonas		25
Euglena viridis		20
Euplotes		5
	<u>Diatoms</u>	
Gomphonema		200
Navicula gracilis		25

Other forms of protozoa and diatoms were present in small numbers, the diatoms being near the surface of the mass.

To determine the number of bacteria present in the *Leptomit* mass, an analysis of some fresh material was made in April, 1916. Here 400,000 colonies per . c. developed on litmus-lactose agar after 48 hours incubation at 37° C. Of these colonies, 10,000 had an acid reaction.

In spring the part of the *Leptomit* growth just above Station 4 is for a time mixed with a luxuriant growth of algae; then, with the advent of warm weather, it rapidly disappears, first at Station 4 and last near Station 2, at places where the current is swift.

In May, 1916, *Leptomit* almost completely disappeared during two weeks of very warm weather when the work of cleaning the surface of the filter beds was begun. During this period, as an examination of Diagram IV will show, the number of bacteria in the stream was greatly increased. Indeed, this was suggested by the appearance of the stream, for in place of the *Leptomit* large gray masses of the stalked Protozoan, *Carchesium*, were everywhere to be seen. Frequent rains, with consequent high stream flow, seem to prolong the period of *Leptomit* growth.

Several factors affect the seasonal distribution of this fungus. Temperature is doubtless the fundamental factor but that the (Page 40) action is not direct is shown by physiological experiments, proving that the optimum temperature for the growth of the plant is approximately 20° C, when other conditions are suitable.*

An examination of the chemical data shows that the food supply in the form of organic nitrogen and albuminoid ammonia is as abundant in summer as in winter, nor are seasonal fluctuations in carbonaceous matter, as expressed by the oxygen consumed figures, related to the growth of the fungus. The slight variation in carbon dioxide is probably coincident rather than causative, inasmuch as it follows the degree of dilution more closely than the amount of *Leptomit* growth, and the mould has been found by Kolkwitz to withstand the acidity of a 0.1 per cent. solution of aspartic acid. It may be noted, on the other hand, that the amount of oxygen dissolved in the water during the period of its growth is greater than six parts per million, while during the summer period it is consistently less than this. The amount of free ammonia also has a corresponding variation, being, roughly, less than six parts per million when *Leptomit* is present and more than eight parts when *Leptomit* is absent. According to other observers, it is more sensitive to alkalies than to acids and free ammonia is not endured in concentrations exceeding eight parts per million.

The foregoing facts seem to associate the growth of the water mould with bacterial activity. It has a high oxygen demand and is readily attacked by putrefactive bacteria or overcome by too great alkalinity in the water. During the summer there is a decrease in dissolved oxygen, a decreased activity of the acid-forming types, an increased activity of those producing alkali and a more intense fermentation of the stream bottom.

*Kolkwitz, R. _____ '03. *Über Bau und Leben des Abwasserpilzes Leptomit* lacteus. Heft 3, Mitteilungen aus der Königlichen Prüfungsanstalt für Wasserversorgung und Abwasser beseitigung zu Berlin, Heft 2

Leptomitius occurs where there is abundant oxygen acting upon varying amounts of carbohydrates and proteins in solution, but not where the activities of ammonifying bacteria are intense. (Page 41)

DIATOMEAE

The seasonal distribution of diatoms in the normal stream corresponds with that usually found, the curve having its highest point in summer but also a high point in winter. Masses of higher algae furnish a suitable abode for diatoms and because of this the distribution of the two types is correlated.

While, because of the presence of filamentous forms such as *Fragilaria*, the individual counts of diatoms vary widely, the yearly average shows a sudden decline in the number at the point where pollution enters the stream. There is a slight increase in this number as far as Station 4 and below the tributary stream the number soon becomes greater than above the point of pollution. It is to be noted that by far the greatest number of diatoms were found in the latter region during the month of September, when the stream flow was lowest and the nitrification was highest.

Doubtless many of the diatoms found at Stations 2 and 3 had been brought from the unpolluted stream, but the fact that the more common forms such as *Navicula*, *Gomphonema*, *Meridion* and to a less extent *Synedra* and *Fragilaria*, occur here consistently and are more abundant at Station 4 shows that the amount of pollution present does not exclude these forms of vegetable life.

In both years of the investigation an early spring increase in the number of diatoms was noted at Station 4, later extending up-stream. This, together with the presence of higher algae, was the first sign of improving conditions.

ALGAE

With the exception of the red alga, *Batrachospermum*, which was found once at Station 6 and once in the small brook above the sewage beds, and the more common form, *Tribonema* (*Conferva*) which is included properly in the group of *Heterocontae*, all of the Algae found belong to the group of *Chlorophyceae*.

In the order *Volvocales*, the form *Volvocis* appeared once,--- in June, 1915, at Station 7 and two other forms, *Eudorina* and *Gonium*, as recorded in the table of microscopical examinations. (Page 42)

Of the *Protococcales*, the most important form is a type of *Protococcus*. This, together with the *Flagellates*, *Chlamydomonas* and *Euglena*, both of which are here classified under *Protozoa*, occurs in the most polluted parts of the stream. These forms have even been found in the raw sewage standing upon the sand beds. *Protococcus* also sometimes occurs with zoogloal masses upon the surface of the trickling filter.

The Sedgwick-Rafter counts do not show the abundance of this form as well as the fact that in the summer of 1915 it appeared frequently on agar plates living symbiotically with a bacillus when samples from Stations 2 to 4 were plated out in the second and third dilution. The table of organisms shows that the other members of this group prefer a clear water habitat.

Two forms not in the above mentioned table are *Palmella*, a specimen of which was collected from the Daley Brook above the filter plant March 10, 1915, and *Tetraspora* which occurred at Station 1 the following April.

Among the Desmids, *Closterium* is by far the most abundant form. This is referred to under two heads in the table of organisms,---*C. Lunula*, which includes two other closely allied forms (*C. moniliferum* and *C. parvulum*), and *C. rostratum*. This genus is commonly found in the polluted region. The other desmids show a decided preference for the clearer water.

Among the filamentous algae, *Spirogyra communis* and *Tribonema* (*Conferva*) are the most abundant and are the ones which grow in the most polluted water. In the months of February and March when the meadow above Station 4 is overflowed, masses of algae made up of these two species, chiefly the latter, cover the whole region, growing with *Leptomitum* and largely supplanting it. On an acre of this inundated meadow the bright green growth is from six to twelve inches deep and may be seen floating on the water in long strands. When the water subsides these algae and the *Leptomitum* form over the grass a black residue which has been mistaken by some for sewage sludge. In the late summer, as the algae become more abundant further up the stream, these two forms are the first to appear and (Page 43) *Tribonema* has been found in a pool of stagnant water caused by an overflow on one of the sand beds.

The other algae in varying degrees appear less able to withstand pollution. *Vaucheria* and *Spirogyra maxima*, although they abound in the normal stream, have never been found between the point of pollution and Station 4.

The genus *Microspora*, not mentioned in the Table, was found but once, in August 1915, at Stations 2 and 5. The list of species gives the names of other algae found and the places where they occurred.

PHANEROGAMS

The higher plants which fill the stream in summer have three important functions. They furnish oxygen to the stream, they retard it, and they act as a roughing or contact filter, upon which suspended particles, including colloidal matter, may accumulate. In the growing season, the pond weed, *Potamogeton*, the most common form in the polluted region, and associated plants, make so efficient a strainer that in August the average turbidity of the water is reduced from 18 to 3 parts per million in the 0.7 mile between Station 2 and 4.

Attempts to determine the velocity of the stream by adding a dye fluorescein, to the water were frustrated because the dye was taken up by the organic matter and strained out by the plant growths. Plants attached, and of some size, also assist in stream purification by reducing the velocity of the current and furnishing shelter for a wide variety of smaller plant and animal forms.

Dr. Edmund W. Sinnott, who spent a day, at our request, on the stream at the height of the growing season, kindly identified the important types of aquatic flowering plants. Nearly all the plants found at all appear in the unpolluted water above the sewage beds. The immediate effect of pollution is to cause them to disappear, but farther down the stream they reappear one by one, many of them within a comparatively short distance, and at Station 4 higher vegetation is more luxuriant than at any other point. A half mile below the affluent stream, vegetation is (Page 44) again normal in quality although more abundant than at Station 1, because of the greater food supply. No attempt has been made to determine particular toxic substances in the polluted water, so that it is impossible to state by what the above mentioned effect is produced.

In the list of species, the higher plants are arranged and numbered in the order of their re-appearance below the sewage effluent drains, and the locations are indicated by these numbers on the map of the stream.

PROTOZOA

Probably no group, other than the bacteria, indicate the chemical condition of the stream as clearly as do the Protozoa. Both by their number and by the kinds found, they signify the degree of pollution.

As one would expect, the Sarcodina are primarily pollution forms, as are most of the Ciliates, while the Flagellates vary from such pollution forms as *Chlamydomonas* and *Euglena*, already mentioned to forms like *Dinobryon* and *Uroglena* rarely found in this part of the stream. The green Flagellates which abound in polluted water probably depend mainly upon carbon dioxide and nitrates for their food supply, although a few may ingest bacteria to some extent. To what degree the Infusoria live upon material other than bacteria and organisms smaller than themselves is not certain but their physiology, in so far as it is known, indicates that the greater part of their food is of the type mentioned. Great numbers of Infusoria have not been found where the bacteria were scarce and, conversely, where bacteria are abundant, as in growths of *Leptomitus*, these Protozoa are plentiful. The cleaning of the filter beds in spring, which causes the bacterial flora of the stream to increase greatly, is also accompanied by an increase in the number of *Carchesium* and other ciliates. Many of these forms never, or only rarely, occur except between Stations 2 and 4.

It is significant to note that the yearly average shows the number of Infusoria to parallel roughly the number of bacteria present (Page 45) at various points in the stream, although, as would be expected, the greatest number of Protozoa are not to be found at the point where the bacteria enter the stream but a short distance below.

The rate of disappearance of bacteria in various zones mentioned in the discussion of the bacterial data, corresponds with the number of Infusoria at the end of each zone even more closely than does the total bacterial count.

In general the Infusoria decrease below Station 3, although this decrease is much more marked in summer than in winter. At Stations 5 and 6 the Protozoa are those of normal streams.

As an indication of pollution, a study of the kinds of Infusoria may be nearly as valuable as an estimation of the number, and further investigation may relate certain types to definite conditions.

The distribution of the many types may best be studied from the table of organisms. Of those indicating pollution, however, the following should be given special mention: the type of Colpidium figured in Plate II, Paramoecium, Metopus, Euplotes, Carchesium, Oxytricha, Lionotus, Loxophyllum, Amoeba, Paramecium, also Euglena and Chlamydomonas when abundant.

Of the above forms, the species of Colpidium is most significant. This is exceedingly common except in late summer and has been found only between Stations 2 and 4. It has not been possible to identify this organism specifically, although it strikingly resembles the Colpidium colpoda of Ehrenberg which has been reported at many sewage purification plants in Europe, as well as the Colpoda cucullus of O. F. Muller and the Colpidium cucullus of Ehrenberg.

The only forms not included in the list of organisms are the Suctorian, Podophrya, found but once at Station 6 in May, 1916; the green Flagellate, Cryosphaerella, occurring in July, 1915, at Stations 5, 6 and 7, and the following Infusoria: Anthrophysa, found but once at Station 2, August 1915; Coleps, found in Daley Brook August 14, 1914; Diffugia occurring once at Station 5, June 1915; Lachrymaria, which occurred in a growth of algae at Stations 1 and 3, August, 1915; and Metopus, (Page 46) found at Station 2, August, 1915, also seen in samples of sewage effluent. These, with the others, are listed in the table of species.

PORIFERA

Sponge spicules occurred consistently in nearly all samples and a survey of the stream bed made in July, 1915, showed that Ephydatia Mulleri (Lieberkuhn) was present in abundance at Station 1, growing in green mats about one inch in diameter on the sides of small rocks in the rapidly flowing regions of the stream. The form was identified by the structure of the seed-like bodies or gemmules and the spicules which they contained. This was the only species present and a comparison showed that the spicules found in the samples were the skeletal spicules of this form. It was not found between Stations 2 and 4, where the soft bottom as well as the increased turbidity would be unfavorable to sponge life, but the number of spicules found at Station 6 showed that it must have made its appearance again in the clear water above that point at places where the bottom was suitable.

COELENTERATA

Innumerable hydrazes of the species *H. oligactis*, Pallas (*H. fuscus*, Linnaeus) occur every fall in the months of October and November on the rank growth of aquatic vegetation in the region of Stations 3A and 4. The "bloom" appears suddenly at the time when higher vegetation is breaking down and when small crustacea have reached their greatest development, disappearing with these at the advent of the colder weather of December. They are to be found attached to all parts of the higher plants and most of them are covered with the ectoparasitic protozoan, *Tricodina pediculus*. Inasmuch as the Hydra is not regarded as a desirable food for fish, but consumes Crustacea which might be used as such, it may be regarded as backward rather than a forward step in the building up of organic matter into higher animal forms. (Page 47)

WORMS

Among the worms, the flatworm *Planaria*, which was found on the higher plants in all parts of the stream, and the ubiquitous round-worm *Anguillula*, are the only representatives of their respective phyla. These are relatively unimportant when compared with some of the Annelids.

Of this last group, the most common form is the red worm *Tubifex* (see Plate I). This is a typical pollution organism and is always to be found between Stations 2 and 4. The worms appear on the muddy bottom in colonies as red patches which vary from three to twelve inches in diameter and contain countless individuals. They live at no great depth of water, being nearest the surface in summer and where the current is least. The head of the worm is in its hole or burrow, while the posterior end, which is used in respiration, waves in the water in search of oxygen. When disturbed they quickly disappear into their tubes.

The presence of haemoglobin in the blood allows the animal to store up oxygen enough to last for some time and like earthworms, which are also occasionally seen in this region, it migrates through the soil of the stream bottom. It not only absorbs considerable organic matter from the material continually passing through its alimentary canal, but gives the pollution carpet a good working over and mixing. These worms form a conspicuous index of the type of bottom laid down by a polluted stream.

The genus *Nais* though not as abundant, is equally constant in its appearance. It occurs, however, in the normal as well as the polluted section of the stream. *Stylaria* was found occasionally, but only where the water was relatively clear.

Of the leeches, five species were found during the survey of the stream in July, 1916. A specimen of *Placobdella hollensis* 40 mm. long and 21 mm. wide was taken from an old branch near Station 3, where a large snapping turtle, which had several smaller specimens attached, was captured a couple of days later. This form was not found elsewhere. *Glossiphonia fusca* was found only on the rocky bottom at Station 1 but the three species (Page 48) mentioned below had a more general distribution.

Of these *Glossiphonia complanata* and *Glossiphonia stagnalis* are reported as feeding upon snails and worms more than upon vertebrates and the still more common *Erpobdella punctata* prefers aquatic insects, bristle worms and larvae.

TROCHELMINTHES

The total number of Rotifera recorded is comparatively small; nevertheless certain facts are evident. There are as many rotifers in the unpolluted as in the polluted parts of the stream and none of these forms are peculiar to the latter region. To be sure, *Rotifer vulgaris*, which has been found in sewage purification plants, is the most common form here. It occurs in the normal stream as well. Thus, although these forms doubtless feed upon bacteria and infusoria they subsist as well upon the lower algae and decaying vegetable tissue and their common appearance where the latter are present is not a proof of pollution.

The list of species includes the following forms not mentioned in the table of organisms: *Conochilus*, the beautiful colonies of which were found attached to plants at Station 3A in August and September, a species of *Dinocharis* found at Station 7, May 1915, and a form of *Pterodina* found at Station 4, October 1915. The gastrotrichan, *Chaetonotus*, appeared in samples from Stations 1, 3A and 5.

MOLLUSCA

Only a few fresh water clams were seen and these were in the normal stream, but the effect of stream pollution in increasing the number of gasteropod molluscs is striking. These snails furnish an obvious proof of increased food supply below the point of pollution; the aquatic plants near Station 3A were covered with them. At this point, one day in August, fifty specimens were gathered by one scoop of the two hands. *Planorbis trivolvis* is the most common, but *Physa* and *Segmentina* are also abundant. In conditions at Station 3A, but one snail, the viviparous *Campeloma*, was found at Station 1. (Page 49)

Although the greatest numbers were found at Station 3A, many were seen in the more quiet water from Station 3 to Station 4, below which the number decreased rapidly. Seasonally they are most abundant in the late summer and fall, but a few less active specimens may be seen throughout the winter.

CRUSTACEA

But three species of Malacostraca have appeared,---the two Amphipods, *Gammarus* and *Hyalella*, which abound in the unpolluted part of the stream, and the Isopod, *Asellus*. *Asellus communis* is in point of size and numbers, the most important crustacean in the stream. It is 15 mm. long by 5 mm. wide and is active everywhere at all times of the year, being most abundant in summer among higher vegetation and in the polluted section. At Station 3A as many as twenty may often be seen on a square foot of stream bottom, while a handful of water-grass or pond-weed may include twice that number.

Smaller animals, corresponding closely to the European species *A. aquaticus* were occasionally found in the clearer water. This species, however, has not been reported south of Labrador and it does not seem probable that it occurs in this region.

Several Entomostraca have appeared at the places indicated in the list of organisms. The genus *Cyclops*, of which four species have been found, is the most common.

An examination of the monthly averages shows that in total numbers the normal stream has as many of these forms as the polluted area and that they are most abundant in the summer and fall, being absent from most samples in the winter. They occur in great numbers in masses of algae and in pools of still water beside the brook. Such a pool just above Station 3 becomes green in summer with *Chlamydomonas* and *Euglena*. Here in the month of August the *Daphnia*-like form *Simocephalus* is so abundant that even the surface water contains more than one thousand per liter. The digestive tract is filled with the green flagellates which appear to constitute their chief food, and it is worthy of note that these forms abound where there are (Page 50) chlorophyll-bearing organisms rather than in the bottom samples rich in filamentous bacteria and colorless protozoa.

Beside the forms mentioned in the table of organisms, the list of species includes two genera, *Canthocamptus* and *Polyphemus*, which were found in special samples.

INSECTA

In its relation to stream purification, no insect is as interesting as the midge, *Chironomus decorus*. Millions of its aquatic larvae, popularly known as "blood worms," are always to be found from the point of pollution to the affluent stream, greedily devouring all sorts of detritus as well as lower plant and animal forms. Nor do these useful larvae become obnoxious insects, as is the case with the mosquito, for the midge does not sting or bite; indeed the mature form probably never feeds.

The stages of development are shown by the accompanying figures (Plate 1, Figs. 8-10). The imago which closely resembles the mosquito, may be readily distinguished from the latter by its habit of raising the fore feet when at rest.

There are many interesting points in its life history. The whole development takes place in the current of the stream. The gelatinous egg sacs, which prevent the eggs from being attacked by bacteria and moulds, or eaten by birds, are attached to a rock or plant at the surface of the stream by means of a small cord which runs through the center. Each sac contains from twelve hundred to fifteen hundred eggs and so profusely are they distributed that a railroad tie which was found partly submerged in the water furnished a hatching place for over 300,000 midges.

The larva is hatched as a white wiggler nearly 1 mm. in length, which soon settles to the bottom and begins to eat, growing in three or four days to one-fourth of an inch in length and taking on a pink color.

This soon deepens to a brilliant red, and the larva begins to make a case for itself by glueing together the material about it with a jelly-like substance from its salivary glands. In many places on the soft, muddy stream bottom these (Page 51) little mounds, each with an opening at the top, number four or five hundred per square foot. A 30 c. c. sample of bottom mud, collected to a depth of two inches, August 1914, contained 130 of these larvae, which together with their cases, made up one fifth of its volume.

The larval period lasts for from several days to several weeks, depending upon the temperature. The mature larvae are 2.5 cm. in length. In the winter they do not develop to pupae but still remain fairly active although if taken to a warm room their development proceeds rapidly. During the larval period the feeding process is almost continuous, the food consisting of whatever is at hand. They are reported as living upon decaying plant tissue, but those found among the iron bacteria were seen to contain the brown substance in their digestive tract while those living in black muck contained food material of that color. They are even cannibalistic under certain conditions, for when those recently hatched from two egg cases were left in clear water a few days their numbers decreased and the remaining forms grew until there were but three left. In masses of algae in the normal stream, an unidentified species of midge was found which did not contain the haemoglobin in its blood but was white or light green in color. The digestive tract of this form was filled with plant chlorophyll and its otherwise clear larval case was partly filled with bits of *Spirogyra*. Thus these insects appear to be real scavengers, the chitinous lining of their digestive tract allowing them to eat even sandy detritus and digest whatever food material it may contain.

When the *Chironomus* pupates, it assumes the shape shown in Figure 10, and still active soon goes floating down stream. Finally the pupa case splits along the dorsum and the imago emerges, rests a minute on the floating pupa case, and then flies away. If the good fortune by which it has escaped fish and predatory insects continues and it is able to avoid the dragon-flies and other forms for a day or two, it reproduces and dies. Swarms of these midges have been seen under alder bushes at Station 3, and upon catching some of them, there were found in many instances large (Page 52) females to which males, smaller and often dead, were still attached.

From the omnivorous habits of the larvae, their value as food for fish, and the number of mature insects which leave the water entirely, it is obvious that *Chironomus* is an important factor in the removal of organic matter.

The air-breathing larvae of the club-footed gnat, *Ptychoptera clavipes*, are also important scavengers, having the same habitat as *Chironomus*. They are much larger than the midge but not so plentiful. In 1915 they were first seen at Station 3 on March 16. After about four weeks they disappeared for a time; the second generation appearing June 9. From then on, they occurred in varying numbers until October. The various stages of development are shown in Plate I, Figs. 4 to 7.

The internal anatomy of the pupa is comparatively simple, the most conspicuous structures being the narrow digestive tract and the pair of breathing tubes which extend along the sides of the body and open at the end of the telescope-like tail. The pupa is much more complex and the breathing tubes open at the tip of the elongated antenna.

No other forms are as strictly confined to the heavily polluted water. Of the truly aquatic insects the whirligig, *Dineutus*, and the water skater, *Hydrotrechus*, are always to be seen; water boatmen, *Corixa* and *Notonecta*, are frequently found below Station 3, while the giant water bug, *Belostoma*, and the water scorpion, *Nepa*, were observed at Stations 3 and 4, respectively.

Aquatic larvae other than those already mentioned were more rarely seen. In making a survey of the stream in August 1916, the larvae of the May-fly, *Baetis*, the Stone-fly, *Perla*, the Dragon-fly, *Libellula*, and certain unidentified Caddis-fly larvae, were found only in the unpolluted water. Larvae of a water tiger (*Agabus*) were frequently seen below Station 3.

In a sample of bottom mud from Station 3 there was found a tipulid larva, possibly that of *Dicranota*. This larva,---reported by Miall as feeding upon *Tubifex*,---remains buried in the mud the greater part of the time. (Page 53)

Two larvae of the Black-fly, *Simulium* were seen. The first was collected together with several *Chironomus* larvae, in a sample of *Leptomitus* taken from Daley Brook below a sewage effluent channel on February 1, 1915. A long worm, which had apparently entered the larva through the anal opening, extended nearly the entire length of the animal. Both the worm and the host were alive when first examined. A second specimen was obtained from the swift water near Station 2, August 1, 1915.

ARACHNIDA

Two Arachnid forms were discovered by the microscopical examination,---*Macrobiotus*, the water bear, and *Acarina*. The latter was found at Station 4 in a summer sample, and in a mass of *Vaucheria* collected from clear water on March 1st. These forms, as well as a large water spider, thought to be *Dolomedes*, appeared during the special survey of the stream at the places indicated in the list of species.

VERTEBRATA

Comparatively little work has been done with the vertebrates but a few facts seem worthy of note.

The major fish life has not been crowded out of the stream by pollution. Brook minnows are frequently seen in the most polluted region, apparently feeding upon the furry growths of *Carchesium*. In the spring suckers are to be seen throughout this region and sometimes brook trout and brook pickerel are found, although this is not their usual habitat.

Several have been seen at Station 4 and above the sewage purification works, so that those found between these points were thought to be passing through the zone of greatest pollution.

Two species of frogs have been found in the stream,---the Green-frog, *Rana clamata*, which is most common in the polluted section, and the Pickerel-frog, *Rana palustris*, which prefers the clearer water.

The group of reptiles is represented by two species of turtles: *Chelodra serpentina* the snapping turtle, which has been found in the (Page 54) muddy bottom between Stations 2 and 4, and *Emys guttata*, the gingerbread turtle, which has been found in the unpolluted stream and at all points below Station 4. In the latter region, they are very abundant. Water snakes have been seen occasionally.

No special attention has been given to the bird life, although its abundance in the valley for a half mile below the sewage beds could not fail to be appreciated. A pair of wild ducks made their home near Station 3A in summer and the robins, bobolinks, grackles, blackbirds, sparrows and crows are always to be seen at this season.

SUMMARY LIST OF SPECIES

Showing Sampling Stations at or near which they were found. Stations where forms occur with relative infrequency are inclosed in parentheses.

PLANTS

CRYPTOGAMS.

SCHIZOPHYTA.

CYANOPHYCEAE.

<u>Species</u>	<u>Stations</u>
<i>Oscillatoria percurssa</i> , Kutz	All
<i>Oscillatoria subtilissima</i> , Kutz	2, 4, 6

SCHIZOMYCETES (Higher Bacteria)

<i>Beggiatoa</i> sp.	All
<i>Cladotrix</i> (<i>Sphaerotilus</i>) sp	2, 3, 3A, 4, (5), (6)
<i>Crenothrix polyspora</i> Cohn	2, 3, 3A, 4

EUMYCETES. PHYCOMYCETES.

<i>Leptomitius lecteus</i> Agardh	2, 3, 3A, 4 (Page 55)
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DIATOMAEAE

<i>Amphora</i> sp.	1, (3), (3A), 5, 6, 7
<i>Cyclotella operculata</i> Kutz	1, (2), (3), 4, 5, 6, 7
<i>Cymbella cistulla</i> Hemprich	1, (2), 5, 6

<u>Species</u>	<u>Stations</u>
<i>Diatoma vulgare</i> Bory	1, 5
<i>Eunotia</i> (<i>Himatidium</i>) <i>major</i> Smith	1, 3, 4, 5, 6
<i>Fragilaria</i> sp.	All
<i>Gomphonema geminatum</i> Agardh	All
<i>Meridion circulare</i> Agardh	All
<i>Navicula gracilis</i> Ehrb.	All
<i>Navicula parva</i> Ehrb.	All
<i>Navicula rhynchocephala</i> Kutz	All
<i>Navicula viridis</i> Ehrb.	All
<i>Pleurosigma</i> sp.	1, (2), 5
<i>Stauroneis</i> sp.	1, 2, 3, 3A
<i>Surirella splendida</i> Ehrb.	1, 3, 3A, 4, 6
<i>Synedra pulchella</i> Kutz	1, 3, 5, 6, 7
<i>Synedra ulna</i> Ehrb.	All
<i>Tabellaria fenestrata</i> Kutz	All
<i>Tabellaria floccuosa</i> Kutz	All

EUPHYCEAE. CHLOROPHYCEAE. VOLVOCALES.

<i>Eudorina elegans</i> Ehrenberg	1, 5
<i>Gonium pectorale</i> Muller	1, 3, 3A, 4, 5, 6
<i>Volvox globator</i> Ehrenberg	7

PROTOCOCCALES.

<i>Ankistrodesmus</i> (<i>Raphidium</i>) <i>falcatus</i> Corda	1, 6
<i>Palmella mucosa</i> Kutz	1
<i>Pediastrum Ehrenbergii</i> A.Br.	1, 3A, 4, 5
<i>Protococcus</i> sp.	All
<i>Scenedesmus caudatus</i> Corda	1, 3, 4, 5, 6, 7
<i>Tetraspora gelatinosa</i> Desv.	1

(Page 56)

CONJUGALES. DESMIDS.

<i>Closterium lunula</i> Ehrb.	1, 4, 5
<i>Closterium moniliferum</i> Ehrb	All
<i>Costerium parvulum</i> Nag.	All
<i>Costerium rostratum</i> Ehrb.	All
<i>Cosmarium Broomei</i> Thwaites	4, 5
<i>Hyalotheca dissiliens</i> Breb.	1, 4, 5, 6, 7
<i>Penium closteroides</i> Ralfs	All
<i>Staurostrum</i> sp.	All

ZYGEMACEAE.

<i>Spirogyra communis</i> Kutz	All
<i>Spirogyra maxima</i> Witt	1, 4, 5, 6, 7
<i>Zygnema</i> sp.	1, 4, 5, 6, 7

SpeciesStations

ULOTRICHALES.

Chaetophora sp.	1, 5, 6
Cladophora sp.	1, 4, 5, 6, 7
Draparnaldia sp.	4, 5
Microspora stagnorum Lag.	2, 5
Stigeoclonium sp.	2, 3A, 4, 5, 6, 7
Ulothrix sp.	1, 4, 5

SIPHONALES.

Vaucheria geminata Vauch.	1, 5, 6, 7
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HETEROCONTAE.

Tribonema (Conferva) bombycinum Derbes & Sol.	All
Tribonema (Conferva) minus Haz.	All

RHODOPHYCEAE.

Batrachospermum sp.	1 (Page 57)
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PHANEROGAMS

The flowering Plants are listed in the order of their reappearance below the point of pollution. The same numerals are used on the map to indicate where the plants reappear.

Stations

1. Potamogeton epihydrus (Pondweed)	All
2. Sparganium americanum, var. androcladum (Burr reed)	1, 3, 3A, 4, 5, 6
3. Sagittaria latifolia (Arrowhead)	1, 3, 3A, 4, 5, 6
4. Polygonum hydropiper (Water pepper)	1, 3, 3A, 4, 5, 6
5. Glyceria pallida (A common water grass)	1, 3, 3A, 4, 5, 6
6. Cellitriche heterophylla (Water starwort)	1, 3A, 4, 5, 6
7. Pontederia cordata (Pickerel weed)	1, 3A, 4, 5, 6
8. Ludwigia palustris (Water purslane)	1, 3A, 4, 5, 6
9. Mentha avensis (Corn mint)	1, 4, 5, 6
10. Bidens sp. (Burr marigold)	1, 4, 5, 6
11. Scirpus validus (Bullrush)	1, 4, 5, 6
12. Glyceria canadensis (Rattlesnake grass)	1, 4, 5, 6
13. Sium cicutifolium (Water parsnip)	1, 4, 5, 6
14. Typha latifolia (Cat-tail)	1, 4, 5, 6
15. Solidago canadensis (Golden rod)	1, 4, 5, 6
16. Lemna (Duck weed)	1, 4, 5, 6
17. Chelone glabra (Turtle head)	1, 5, 6
18. Zizania palustris (Water rice)	1, 5, 6
19. Nuphar advena (Yellow pond lily)	1, 5, 6
20. Mimulus ringens (Monkey flower)	1, 5, 6
21. Lobelia cardinalis (Cardinal flower)	1, 5, 6
22. Radicula Nasturtium-aquaticum (Water cress)	1, 5, 6
23. Ranunculus aquatilis, var. capillaceus (Water buttercup)	1, 5, 6
24. Veronica scutellata (Marsh speedwell)	1, 5, 6
25. Elaeocharis tenuis (Spike rush)	1, 5, 6

	<u>Stations</u>
26. <i>Hypericum</i> sp. (St. John's wort)	1,5,6
27. <i>Juncus canadensis</i> (common rush)	1,5,6
28. <i>Gratiola aurea</i> (Hedge hyssop)	1,5,6 (Page 58)

ANIMALS

PROTOZOA. SARCODINA.

<u>Species</u>	<u>Stations</u>
<i>Actinopshrys</i> sol Ehrb.	3,3A,4,5,6
<i>Amoeba</i> sp.	2,3,3A,4
<i>Arcella vulgaris</i> Ehrb.	All

SUCTORIA.

<i>Podophrya</i> sp.	6
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ASTIGOPHORA. CHLOROPHYLACOUS FLAGELLATES.

<i>Chlamydomonas</i> sp.	All
<i>Chloramoeba</i>	2,3A,6
<i>Cryptomonas</i> sp.	1,2,3,4
<i>Cryosphaerella longispina</i> Lauterborn	5,6
<i>Dinobryon sertularia</i> Ehr.	1,(3),(3A),4,5,6
<i>Euglena oxyuris</i> Schm.	1,2,3,3A,4,5
<i>Euglena spirogyra</i> Ehr.	1
<i>Euglena viridis</i> Ehr.	All
<i>Mallomonas</i> sp.	6,7
<i>Phacus pleuronectes</i> O. F. M.	2,3,3A,4,6
<i>Synura uvella</i> Ehrb.	All
<i>Trachelomonas</i> sp.	1,2
<i>Uroglena americana</i> Calk.	1,4,5
<i>Uvella</i> sp.	1,3,4,5

DINOFLAGELLATA

<i>Glenodinium</i> sp.	All
<i>Peridinium tabulatum</i> Ehr.	All

COLORLESS FLAGELLATES.

<i>Chilomonas paramecium</i> Ehrb.	1,5
<i>Monas</i> sp.	All
<i>Peranema</i> sp.	1,3,5 (Page 59)

INFUSORIA

<u>Species</u>	<u>Stations</u>
Anthophysa vegetans Stein	3
Bursaria sp.	3,4,5
Carchesium polypinum Linn	2,3,3A,4,(5),(6)
Coleps sp.	6
Colipidium Colpoda Ehr. (?)	2,3,3A,4
Diffugia sp.	5
Dileptus monilatus Stokes	1,2,3
Dileptus gigas C. & L.	2,3
Euplotes sp.	2,3A
Lachrymaria olor Mull	1,2,3
Lionotus sp.	2,3
Loxophyllum sp.	2,3,3A,4,(5)
Metopus sigmoides C. & L.	2
Oxytricha sp.	2,3,3A,(6)
Paramoecium sp.	2,3,3A,4,(5),(6)
Rhabdostyla sp.	1,3,3A,4,5,6
Spirostomum ambiguum Ehrb.	3,4,5
Stentor sp.	3,4
Trichodina pediculus Ehrb.	3A,4
Vorticella sp.	All

ROTIFERA.

Ephydatia Mulleri Lieberkuhn	1,5,6
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COELENTERATA.

Hydra oligactis Pallas (H. fusca Linn)	3A,4
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PLATYHELMINTHES.

Planaria albissima Vejdovsky	1,3,5,6
Planaria lugubris Schm.	1,3,4,5,6

NEMATHELMINTHES.

Anguillula sp.	All (Page 60)
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ANNULATA.

Lumbricus sp.	3,3A,4
Nais sp.	All
Stylaria sp.	1
Tubifex tubifex Mull.	2,3,3A,4

HIRUDINEA.

Erpobdella punctata Moore	1,3,3A,4,5
Glossiphonia complanata Linn.	1,4,5
Glossiphonia fusca Castle	1
Glossiphonia stagnalis Linn.	1,3,3A,4,5
Placobdella hollensis Whitman	3

<u>Species</u>	<u>Stations</u>
TROCHELMINTHES. ROTIFERA.	
Anuraea sp.	All
Brachionus sp.	2,3A,5
Conochilus volox Ehrb.	3,3A,5
Diglena sp.	All
Dinocharis sp.	7
Monostyla lunaris Ehrb.	1,3A,4,5,6
Polyarthra sp.	3A,4,6,7
Pterodina sp.	4
Rotifer vulgaris Shrank	All
GASTROTRICHA	
Chaetonotus latus Mill.	1,3A,5
MOLLUSCA.	
<u>Acephala</u>	
Unio complanata Solander (Fresh-water mussel)	1,5,6
<u>Gasteropoda</u>	
Campeloma decisum Say	1
Physa heterostrophia Say	3A,4,5,6
Segmentina armigera Say	3A,4
Planorbis trivolvis Say	3,3A,4,5,6 (Page 61)
ARTHROPODA. CRUSTACEA. MALACOSTRACA.	
Asellus communis Say	All
Hyalolella knickerbockeri Bate	1,(3A),5,6
Gammarus sp.	5,7
ENTOMOSTRACA.	
Canthocamptus northumbricus, Brady	4,5,7
Cyclops albidus Jurine	3A,4,5
Cyclops aquoreus Fischer	2,3
Cyclops fimbriatus Fischer	2,3
Cyclops serrulatus Fischer	All
Cypris sp.	4,5,6,7
Pleuroxus denticulatus Birge	2,4,6,7
Polyphemus sp.	7
Scapholeberis mucronata Mueller	3,3A,4
Simocephalus serrulatus Koch	1, 3, 3A, 4, 5,6,7
INSECTA.	
Agabus sp. (Water-tiger)	3A,4,5
Belostoma flumineum Say	3
Baetis (Ecdyurus) fluminum (May-fly)	1
Caddis-fly larva sp.	1,5,6
Chironomus decorus Johann (Midge)	2,3,3A,4

<u>Species</u>	<u>Stations</u>
Corixa sp. (Water boatman)	3,3A,4,5,6
Culex sp. (Mosquito)	2,3,3A,4
Dineutus vittatus Germ. (Whirligig)	All
Gerris (Hydrotrechus) remigis Fabr. (Water skater)	All
Libellula (Dragon Fly)	1
Nepa apiculata Uhler (Water Scorpion)	4
Notonecta Uhleri Kirk (Water boatman)	2,3A,4,5
Perla sp. (Stone Fly)	1
Ptychontera clavipes Fabr. (Club-footed gnat)	3,3A
Simulium sp. (Black fly)	2
Tipulid larva sp.	3 (Page 62)

ARACHNIDA.

Acarina sp.	1,4
Dolomedes sp.	3,3A,4
Macrobiotus sp.	1,4,5,6

VERTEBRATA. PISCES.

Boleosoma olmstedii Storer (Tessellated Darter)	1,3,3A,4,5,6
Catostomus commersonii Lacepede (Sucker)	All
Esox americanus Gmelin (Brook pickerel)	3,4,5,6
Notropis bifrenatus Cope (Bridled minnow)	All
Salvelinus fontinalis Michill (Brook trout)	1,3,3A,4,5,6

AMPHIBIA.

Rana palustris Le Conte (Pickerel frog)	1
Rana clamata Daudin (Green Frog)	2,3,3A,4

REPTILIA.

Cheledra serpentina (Linne) (Snapping Turtle)	3,3A,4
Clemmys guttata Strauch (Spotted turtle)	1,4,5,6 (Page 63)

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PLATES

PLATE I.

Drawings from Living Organisms.

- Fig. 1. *Tubifex tubifex* Muller, x 6.
- Fig. 2. *Chlamydomonas* sp. x 1500.
- Fig. 3. *Euglena viridis* Ehr. x 1000.
- Fig. 4. Larva of *Ptychoptera clavipes* Fabr. x $1\frac{1}{2}$.
- Fig. 5. Head of *Ptychoptera* larva x 15.
- Fig. 6. Pupa of *Ptychoptera*, Ventral view, x $1\frac{1}{2}$.
- Fig. 7. Head of *Ptychoptera* pupa, Dorsal View, x 4.
- Fig. 8. Larva of *Chironomus decorus* Johann, Lateral View, x 5.
- Fig. 9. Head of *Chironomus* larva, Dorsal View, x 10.
- Fig. 10. Pupa of *Chironomus*, Lateral View, x 5.

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PLATE II.

- Fig. 11. *Ptychoptera clavipes* Fabr. Mature form x $1\frac{1}{2}$.
- Fig. 12. End of Egg Sac of Midge, *Chironomus decorus* Johann, showing rows of eggs, x 5.
 - b. Lateral view of egg much enlarged
 - c. Ventral view of egg.
- Fig. 13. *Hydra oligacta* Pallas x 15. Stained specimen showing many protozoa, *Tricodina pediculus*, attached. Photo by H. C. Lithcoe, '95.
- Fig. 14. *Gammarus neglecticus*. x 3 (After Gerstaecker)
- Fig. 15. *Scaphoeberis mucronata* Mueller. x 30 (After Herrick)
- Fig. 16. *Pleuroxus denticulatus* Birge, Male. x 40 (After Herrick)
- Fig. 17. *Asellus aquaticus* Linn. x 4. (After Gerstaecker)
 - a. Dorsal view.
 - b. Ventral view.
- Fig. 18. *Spironstomum ambiguum* Ehr. Living specimen x 400.
- Fig. 19. *Colpidium colpoda* (?) Ehr. Dorsal view of form fixed in sub-acetic acid x 400.
- Fig. 20. *Colpidium colpoda* (?) Ehr. Lateral view of living specimen x 400.
- Fig. 21. *Loxophyllum rostratum* (?) Ehr. Living specimen x 300.
- Fig. 22. Habit sketch of *Carchesium* growth x $2\frac{1}{2}$.
- Fig. 23. Habit sketch of a bit of *Leptomitrus* x $\frac{1}{2}$.
- Fig. 24. *Leptomitrus lacteus* Agardh x 500.
 - a. Young tip
 - b. Region of branching.
 - c. Young zoosporangium.
 - d. Zoospores.

PLATE III.

Figures after H. W. Conn.

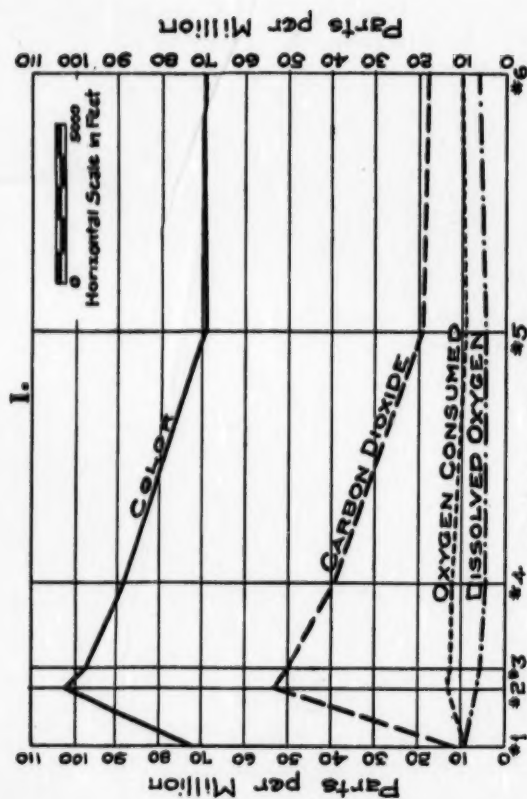
- Fig. 25. *Dileptus monilatus* Stokes x 225.
- Fig. 26. *Dileptus gigas* (?) C. & L. x 225.
- Fig. 27. *Bursaria truncatello* Mull. x 100.
- Fig. 28. *Stentor caeruleus* Ehr. x 250.
- Fig. 29. *Stentor polymorphus* Ehr. x 225.
- Fig. 30. *Paramoecium bursaria* Ehr. x 250.
- Fig. 31. *Paramoecium bursaria* x 250.
- Fig. 32. *Paramoecium trichium* Stokes x 250.
- Fig. 33. *Oxytricha fallax* Stein x 250.
- Fig. 34. *Vorticella* x 250.
- Fig. 35. *Vorticella* x 250.
- Fig. 36. *Lacrymaria olor* C. F. Mull. Fig. 36a is the same animal contracted. x 250.
- Fig. 37. *Paramoecium caudatum* Ehr. x 250.
- Fig. 38. *Oxytricha bifaria* Stokes x 250.
- Fig. 39. *Oxytricha bifaria* Stokes x 250.

PLATE IV.

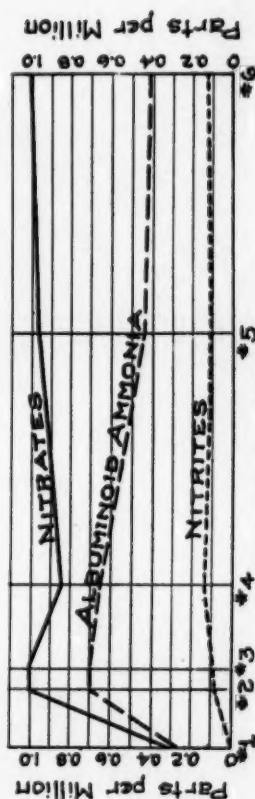
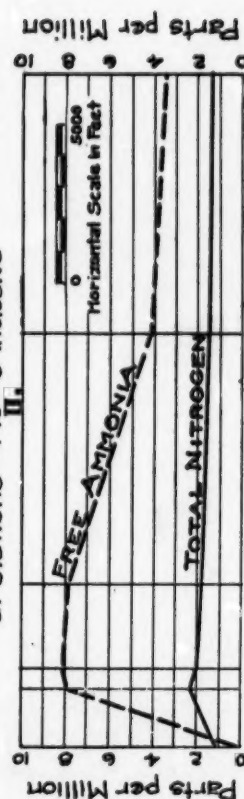
Figures after H. W. Conn.

- Fig. 40. *Carchesium polypinum* Linn.
a. is an expanded colony.
b. is a piece of a single branch magnified
so as to show the ending of the individual
muscles at the base of each stalk.
c. represents two individuals magnified 500
diameters.
- Fig. 41. *Podophrya* sp. (?) showing terminal cilia x 500.
Fig. 42. *Vorticella* x 500.
Fig. 43. *Euplotes plumipes* Stoll x 500.
Fig. 44. *Oxytricha pellionella* Mull. x 500.
Fig. 45. *Trichodina pediculus* Ehr. x 500.
Fig. 46. *Euplotes* sp. (?) x 500.
Fig. 47. *Metopus sigmoides* C. & L. x 500.
Fig. 48. *Rhabdostyla brevipes* (?) C. & L. x 500.
Fig. 49. *Lionotus Wrzesmowsky* S. K. x 500.

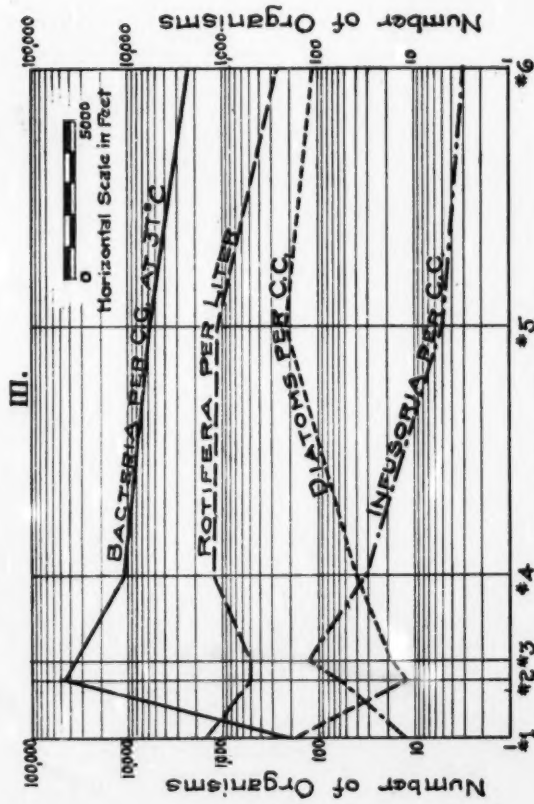
See p. 16



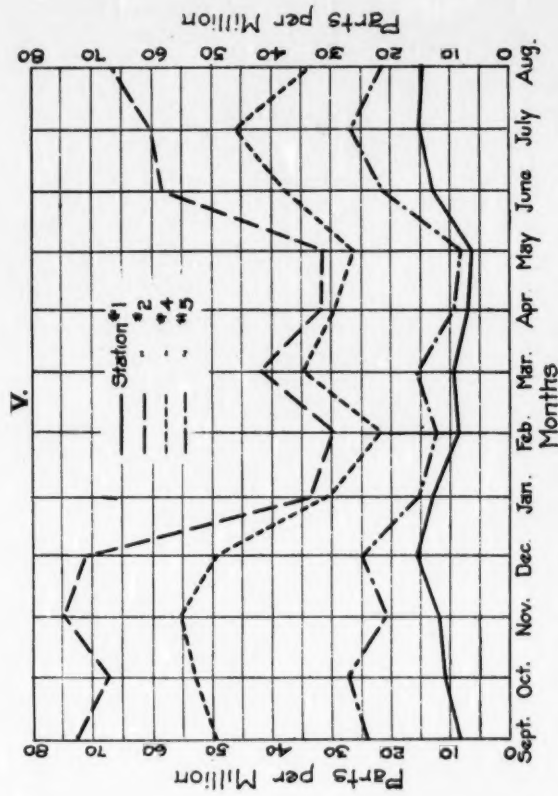
COLOR, CO₂, O₂ CONSUMED AND DISSOLVED O₂ at Stations #1 to #6 Inclusive



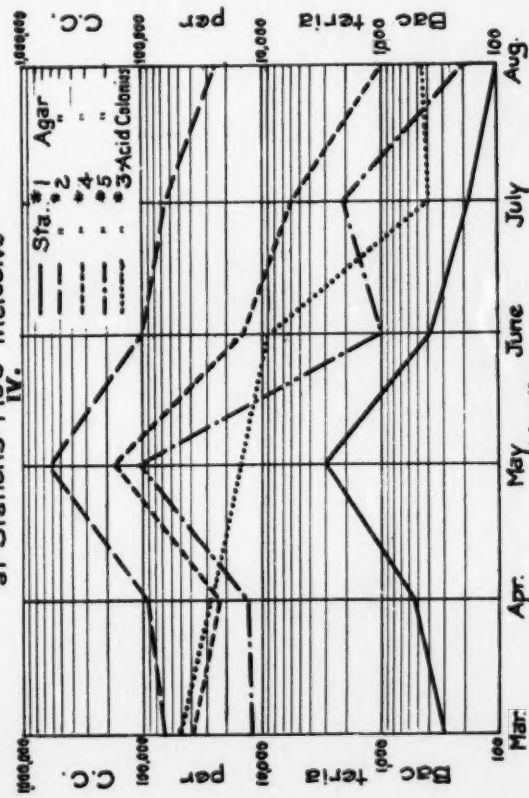
NITROGEN AS FREE NH₃, ALBUMINOID NH₃, NITRATES, NITRATES AND TOTAL N at Stations #1 to #6 Inclusive



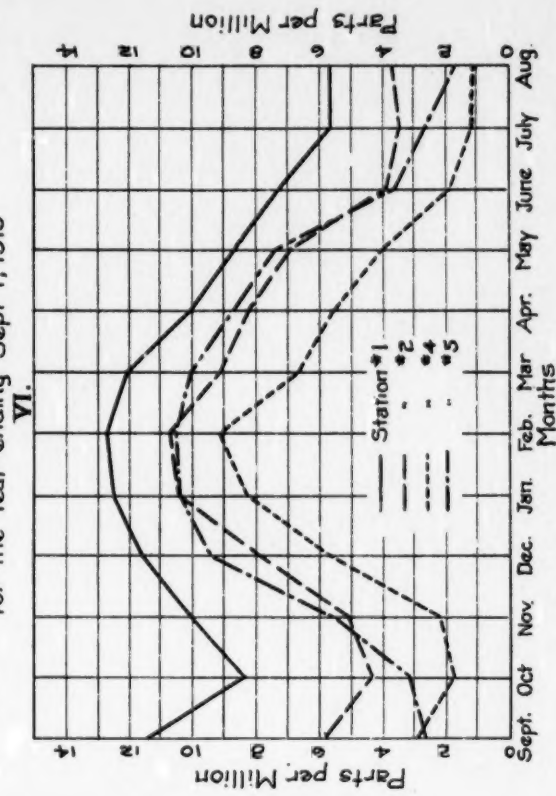
Average Monthly CARBON DIOXIDE at Stations 124*5
for the Year ending Sept 1, 1915



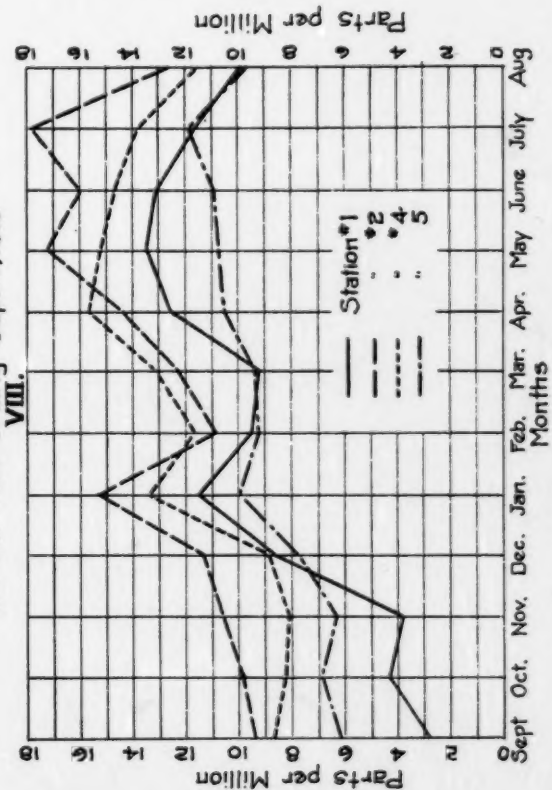
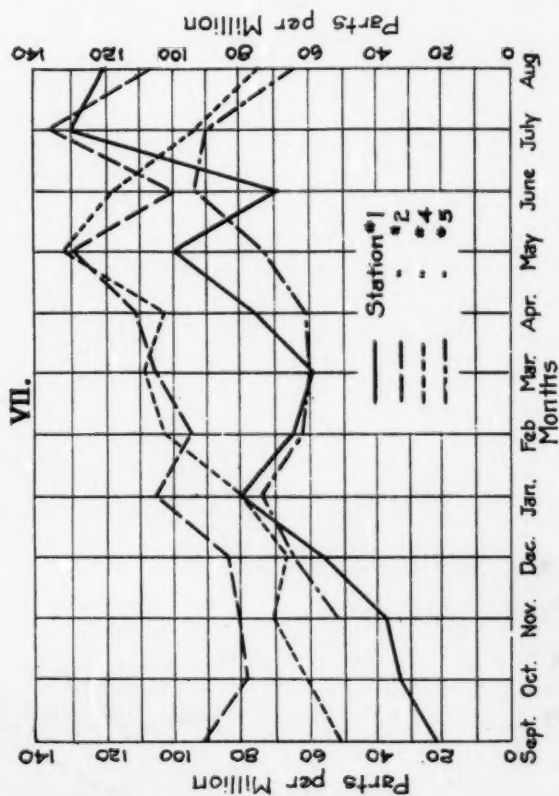
Average Monthly DISSOLVED OXYGEN at Stations 124*5
for the Year ending Sept 1, 1915



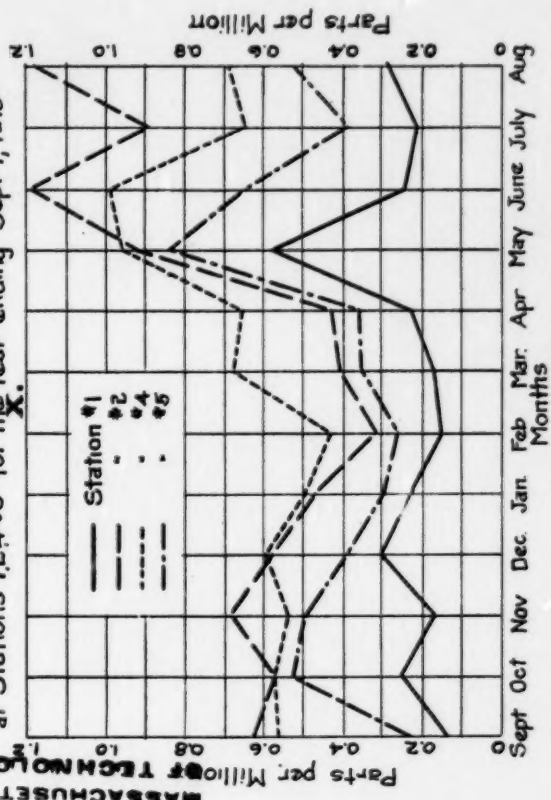
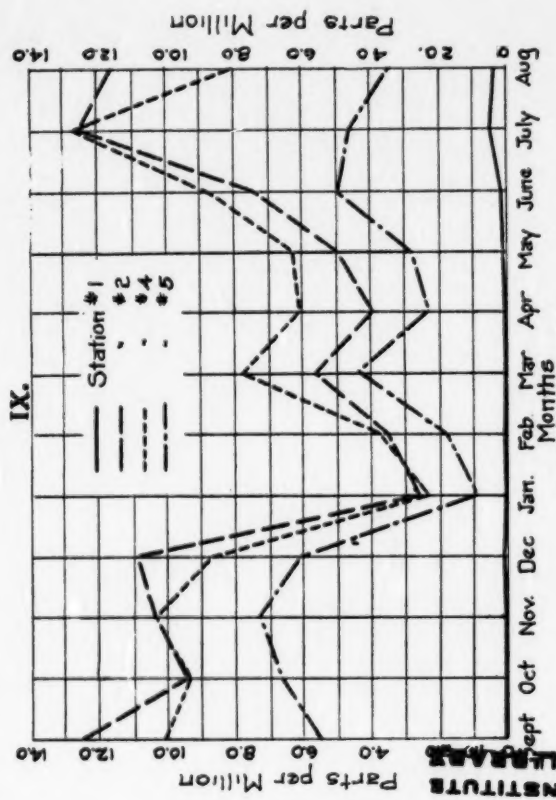
Average Monthly
TOTAL BACTERIA AND ACID COLONIES ON AGAR
for the 6 Months ending Sept. 1, 1915



Average Monthly DISSOLVED OXYGEN at Stations 124*5
for the Year ending Sept 1, 1915

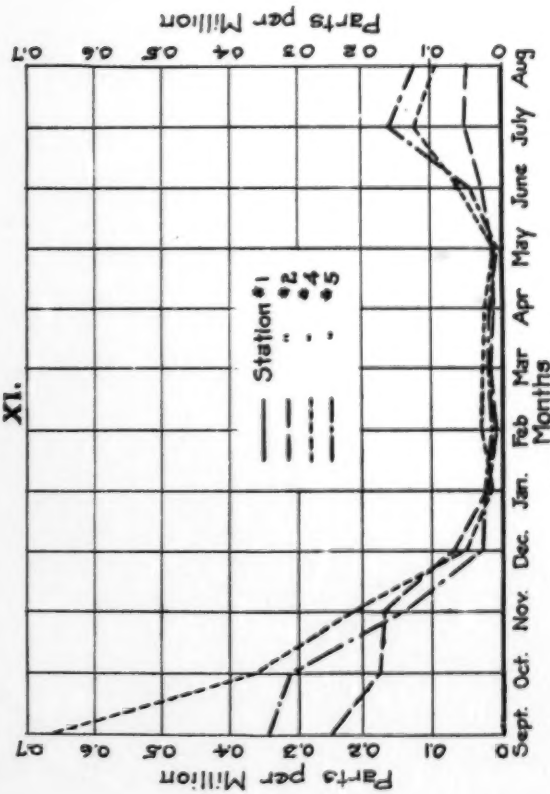


(28)



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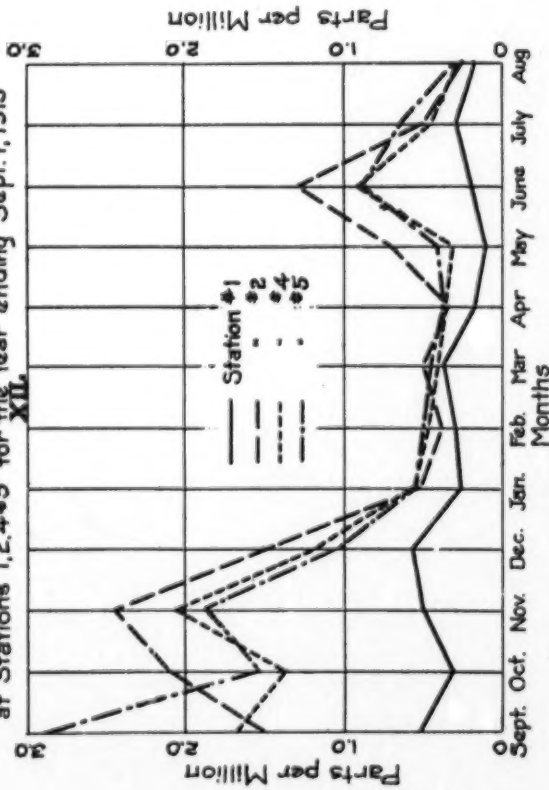
XI.



Average Monthly

NITROGEN AS NITRATES
at Stations 1,2,4+5 for the Year ending Sept. 1, 1915

XII.

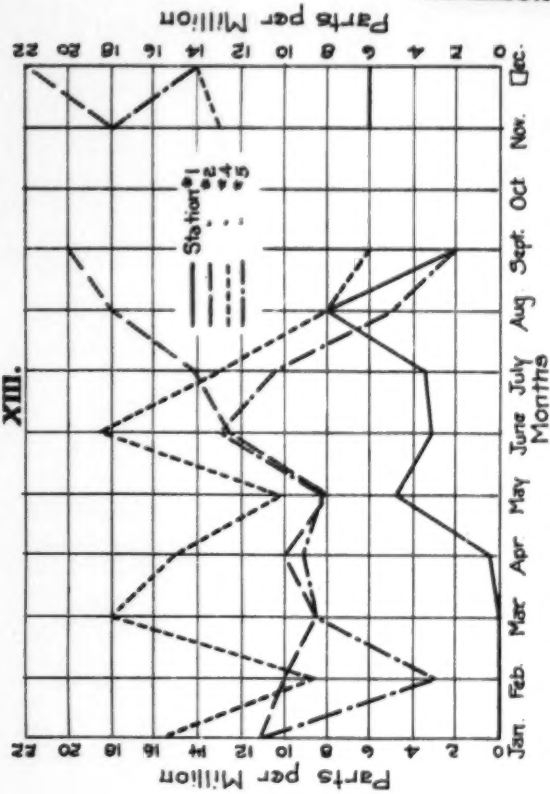


Average Monthly

NITROGEN AS NITRATES
at Stations 1,2,4+5 for the Year ending Sept. 1, 1915

(30)

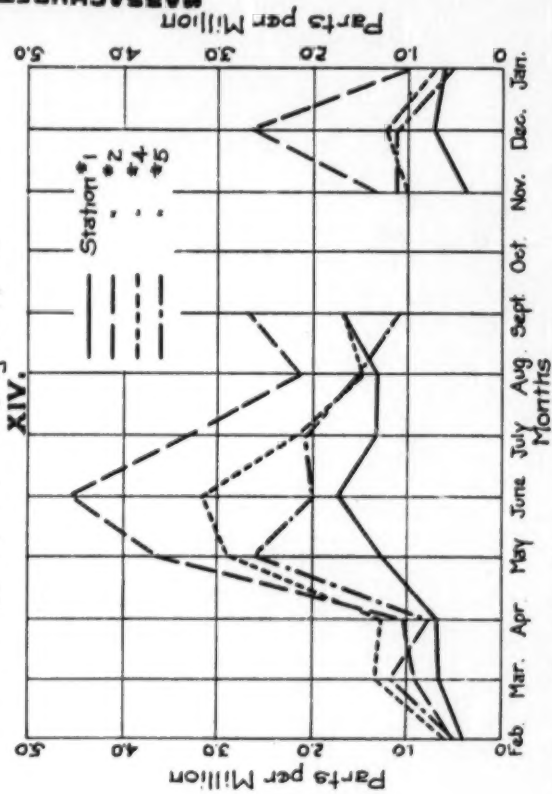
XIII.



Average Monthly

TURBIDITY at Stations 1,2,4+5
for the Year ending Jan. 1, 1916

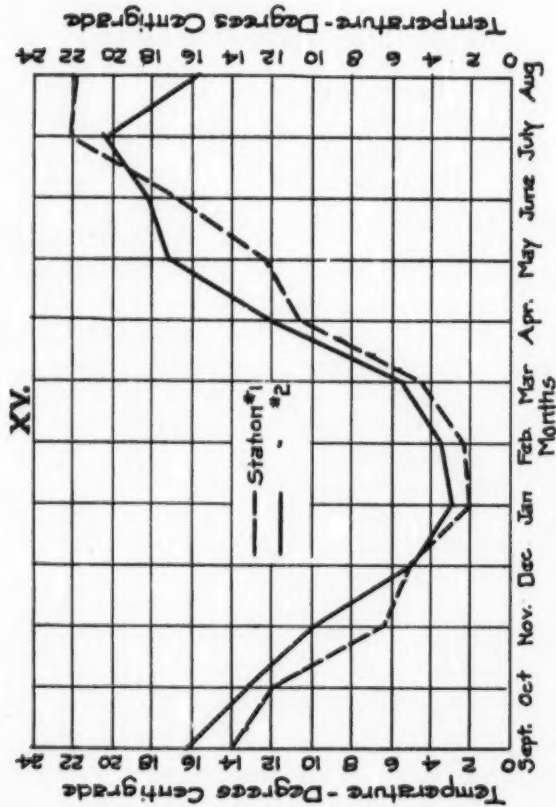
XIV.



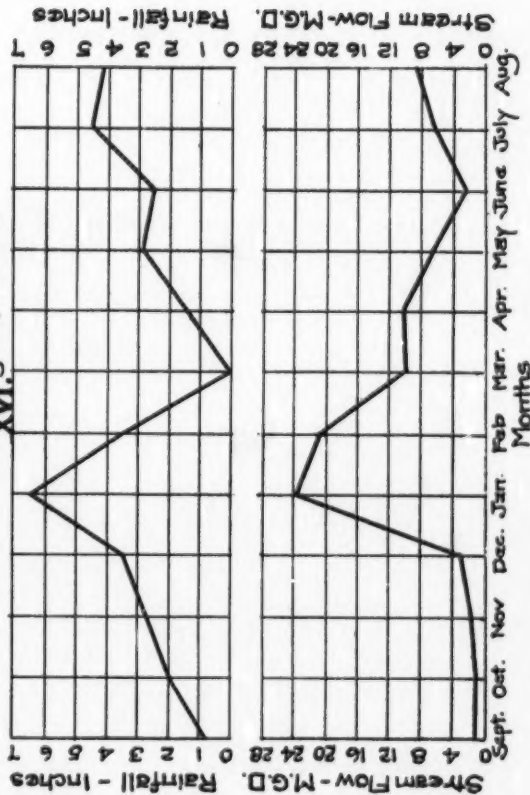
Average Monthly

ORGANIC NITROGEN at Stations 1,2,4+5
for the Year ending Feb. 1, 1916

(31)



Average Monthly
TEMPERATURES OF STREAM AT STATIONS #1 AND #2
for the Year ending Sept. 1, 1915



Average Monthly
STREAM FLOW AND TOTAL RAINFALL
for the Year ending Sept. 1, 1915